Human Serum Low Density Lipoprotein-Sodium Deoxycholate Interaction

(Received for publication, June 30, 1982)

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The binding of sodium deoxycholate to low density lipoprotein (LDL) is a relatively fast process, as compared to the lipid displacement reaction and solubilization. The initial reaction has a bimolecular rate constant of \( \sim 539 \text{ m}^{-1} \text{ s}^{-1} \). In the presence of 1 mM sodium deoxycholate, below critical micelle concentration, 0.04 g of sodium deoxycholate was bound per g of LDL, resulting in an increase in particle radius from 0.128 ± 0.3 Å and an axial ratio of 5.6 for a prolate ellipsoid. Such increases were also observed using higher sodium deoxycholate concentrations and are apparently due to sodium deoxycholate-induced elongation or distortion of certain portions of LDL.

LDL\(^1\) contains approximately 77% lipid, including cholesterol, cholesteryl ester, phospholipid, triglyceride, small amounts of sphingolipid and carotenoids, and unidentified lipids (1). It is presently characterized as having a density of 1.006 ~ 1.063 g/ml, a particle weight of 2.5 ~ 3.9 \( \times 10^6 \) g/mol, and a spheroid particle diameter of 180 ~ 260 Å (1-5).

Various proposed models for the low density lipoprotein subclass have as a common structural feature a core of cholesteryl ester and triglyceride covered by a layer of polar constituents, apo-B, phospholipid, and cholesterol (2, 5). Several important metabolic processes occur at the interface of these domains or involve the transport of lipids from one domain to another, and the core composition may be related to events occurring at the LDL surface.

Although the secondary structure of apolipoprotein B in the intact LDL particle remains unresolved, a model based on density and composition data suggests that the volume occupied by apo-B can accommodate 30% extended helices and 70% globular (\( \beta \)) structure (2).

Surfactants such as SDS (6, 7, 9), Brij 58 (8), Tween 80 (10, 12), N-dodecyl octaethyleneglycol monocouther (13, 14), Triton X-100 (8-10, 12, 15), sodium deoxycholate (12, 15, 16), and Lubrol WX (8, 17) have been shown to provide a structure-forming environment in which native order conformation of solubilized proteins can be induced (17-22). It is also known that the surfactant micelle can effectively strip the lipid from LDL particles and alter the conformation of protein in solution. Sodium deoxycholate, which is a very mild ionic detergent used for solubilizing membrane-bound proteins without altering the biological activity, is also a natural amphiphile, bearing some structural resemblance to cholesterol and forming small mixed micelles with cholesterol and other LDL lipids. The sodium deoxycholate monomer is small and, used in an appropriate solvent, forms stable micelles above the CMC with a low aggregation number, the viscosity of which changes very little at low temperatures.

In this communication, we examine the efficiency and dynamics of lipid displacement from LDL by sodium deoxycholate at varying concentrations below and above CMC, in scanning molecular sieve chromatography and stopped flow kinetics experiments.

MATERIALS AND METHODS

**LDL Preparation**—Freshly drawn plasma was obtained from the blood bank and 1 ml of a solution containing 0.01% Na\(_2\)EDTA, 0.02% Na\(_2\)S, 0.01% Thimerosal (Sigma Chemical Co.), 0.002% phenylmethylsulfonyl fluoride (Sigma) was added to inhibit potential proteolytic activity per 100 ml of plasma. Soluble KBr was used to adjust the desired background density, and LDL was obtained using two centrifugation runs for 20 h (53,000 rpm, 105,000 × g) at 4 °C each at the 1.019 and the 1.063 g/ml density cuts in order to prepare the lipoprotein free of very low density lipoprotein and high density lipoprotein. In all runs, a 60-Ti rotor (Beckman Instrumenta) was used. The isolated LDL was further fractionated by gel filtration chromatography over Sepharose 6B (Pharmacia Chemicals) as described elsewhere (3).

The solvent systems used in this study were 0.2 M NaHCO\(_3\) (Mallickrodt), 0.02% Na\(_2\)S, pH 8.5 and 9.7 (8.5 and 9.7 buffer), ionic strength of 0.2. In some cases, the 8.5 buffer contained 0.01% Thimerosal, 0.01% EDTA, and 0.002% phenylmethylsulfonyl fluoride. Sodium deoxycholate was obtained from Schwarz/Mann (No. 804312) and was used without further purification.

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis**—Low density lipoprotein and LDL-sodium deoxycholate complexes were electrophoresed on 5% polyacrylamide gels in the presence of 0.1% SDS and 0.1 M Tris, 0.2 M sodium acetate, and 20 mM EDTA, pH 7.4 (electrophoresis buffer), according to the method of Weber and Osborn (23). Samples were prepared by heating to 55 °C and for 1 h (unless otherwise specified) in the presence of 1% SDS and 1% \( \beta \)-mercaptoethanol in electrophoresis buffer. The gels were fixed overnight in 50% methanol, 9% acetic acid and then stained with 0.125% Coomassie brilliant blue R-200 dye in fixing solution. The gels were scanned at 590 nm using a Beckman model 24 scanning spectrophotometer and the relative mobilities of the bands on each gel were determined from the corresponding peaks. The relative amount of protein in all bands was determined by estimating the area of each peak from the product of its height times the half-width at the half-height, summing these areas, and then determining the weight fraction of each peak. Major bands were then distinguished as those which constituted more than 15% of the total protein on the gel.
Chemical Assays—The protein content of all samples in milligrams/ml was determined by the method of Lowry et al. (24), using human serum albumin fraction V (Calbiochem Corp.) as a standard. The concentration of LDL was taken to be 4.35 times the protein concentration in milligrams/ml, assuming the protein content in LDL to be 23% (2).

Sedimentation Equilibrium Measurements—All sedimentation equilibrium experiments were performed with a Beckman model E analytical ultracentrifuge equipped with a rotor temperature indicator control unit at 20 °C, using an Yphantis (25) three-channel cell with a carbon-filled epoxy centerpiece in an An-D rotor at 5227 rpm.

The calculation of the molecular weight distribution and the change in C versus X' were based on the Yphantis (25) method, using a computer program which can be modified for use with an Amdahl 470 v/6 II unit (modified IBM 360/1800, University of Florida’s CIRCA computing facilities), plotting ln J or ln C as a function of X'. The ln C versus X' data were fitted to a least squares polynomial and the values of (d ln C/dx') were calculated by a modification of the sliding three-point least squares quadratic treatment of Yphantis.

The preferential interaction term, which in the present case is a measure of the degree of sodium deoxycholate binding to LDL, may be evaluated for a three-component system at sedimentation equilibrium from the concentration distribution of LDL (C2), as described by the expression

\[ M_3 (d\rho/dC) = (d\ln C/dx') 2RT/\omega^2 \]

where \( M_3 \) is the molecular weight of LDL, \( (d\rho/dC) \) is the change in solution density with changing concentration of LDL, \( (d\ln C/dx') \) is the slope of a plot of ln C versus X', X is the radial distance of each fringe from the center of rotation, R is the gas constant in J/(mol·K), T is the absolute temperature, and \( \omega \) is the angular velocity in radians/s. The extent of the preferential interaction, derived from the three-component theory (26, 27) in terms of the number of grams of sodium deoxycholate preferentially bound per g of LDL, \( \vec{x}_3 \), is given by the relationship

\[ (d\rho/dC) = (1 - \vec{v}_3 \rho_3) + \vec{x}_3 (1 - \vec{v}_3 \rho_3) \]

where \( \rho_3 \) is the solvent density and \( \vec{v}_3 \) and \( \vec{v}_3 \) are the true partial specific volumes of LDL and sodium deoxycholate, respectively.

An alternative approach to determining \( \vec{x}_3 \) from ln C versus X' plots is based on the assumption that any change in the slope (d ln C/dx') of this plot for a solute in the presence of ligand is related to the binding of that ligand. That is,

\[ \vec{x}_3 = (M_3 - M_3) / M_3 \]

where \( M_3 \) and \( M_3 \) are the molecular weights of the LDL, 1 mm sodium deoxycholate complex and LDL alone, respectively. Substitution of \( M_3 \) = 2RT (d ln C/dx')/\omega^2 (1 - \vec{v}_3 \rho_3) and \( \vec{x}_3 = (\vec{x}_3 - \vec{x}_3) / (\vec{x}_3 - \vec{x}_3) \) into Equation 3 and rearrangement gives

\[ \vec{x}_3 = (A_3/A_3) (1 - \vec{v}_3 \rho_3) = (A_3) (1 - \vec{v}_3 \rho_3) / (A_3) \]

where \( A_3 \) and \( A_3 \) are the slopes (d ln C/dx') from linear fits of ln C versus X' plots for the complex with ligand and solute without ligand and \( \rho_3 \) and \( \rho_3 \) are the solvent densities for solvent with and without ligand, respectively.

The effective specific volume may be determined from

\[ \vec{v}_3 = (\vec{v}_3 + \vec{v}_3) / (1 + \vec{x}_3) \]

and the preferential interaction term (42, 43) may be evaluated from

\[ \vec{x}_3 - (\vec{v}_3 \rho_3 - \vec{v}_3 \rho_3) / (1 - \vec{v}_3 \rho_3) \]

Positive values of \( \vec{x}_3 \) indicate preferential binding of sodium deoxycholate, while negative values indicate preferential hydration (28, 42, 43).

Once the true values of molecular weight and \( \vec{v}_3 \) are known, the radius of an equivalent sphere, \( \vec{r}_3 \), may be calculated. The ratio of the radius determined by SMSC over \( \vec{r}_3 \) is equivalent to the frictional ratio of the particle and is a relative measure of particle asymmetry. For frictional ratios grater than 1.0, the particle may be considered ellipsoidal (either prolate or oblate) or even rodlike, and the axial ratio a/b may be determined from f/a according to Van Holde (29).

Scanning Molecular Sieve Chromatography (30–33)—All SMSC experiments were done using a gel column-scanning system which is essentially the same as that described elsewhere (30, 31) with the exceptions of the monochronometer and light source module which are Heath EU-700 and EU 701-50, respectively, as shown in Fig. 1.

The basic operating routine consists of moving a quartz column (24 × 0.90 cm) packed with a suitable gel (i.e. one with minimum scattering) through a horizontally collimated beam of monochromatic light (visible or UV) at a constant rate of 0.19 cm/s for 98 s, during which time transmittance is detected by an Aminco 10-267 solid state, blank-subtract photomultiplier microphotometer through an end-on photomultiplier tube (RCA 6993 for UV or RCA CT164 for visible).

The direct UV gel column scanner built in our laboratory (Olis model 3600, Athens, GA) and the stopped flow (Durrum/Dionex) system are interfaced with a Northstar Horizon 2580 A microprocessor, a Sanyo DM50/2CX video display unit, and an Integral Data Systems model 490 printer/plotter. All of the data presented here were obtained using a record length of 300 data points.

The partitioning properties of varying concentrations of LDL in the presence of ligand were obtained using 70 mL of gel at 0.19 cm/s.
the presence of varying concentrations of sodium deoxycholate, as well as the partitioning properties of sodium deoxycholate itself in Bio-Gel A-5m (Bio-Rad Laboratories, 100-200 mesh, exclusion limit \( \approx 5 \times 10^4 \)), were determined in large zone experiments at 220 nm at 25 \(^{\circ}\)C. In this type of experiment, a sample solution at the desired concentration is added continuously to the column, until a reproducible baseline is obtained.

The column is scanned at regular intervals as the solution/solvent boundary moves through the gel matrix at a constant flow rate of 4.30 ml/h. The baseline records of successive scans are subtracted from each other, yielding difference profiles. The problem of locating the centroid position of each boundary is then reduced to the simple task of locating the peak position of these difference profiles (30, 31).

Partitioning calibration parameters for a given column are obtained by determining the rate of movement of a series of sample macromolecules of known molecular radius (32, 33). The partition coefficient is calculated from

\[
s = \frac{(dt/dx)_{a} - (dt/dx)_{b}}{(dt/dx)_{a} - (dt/dx)_{b}},
\]

where \((dt/dx)_{a}\) is the slope of a plot of time versus centroid position for a given sample marker, \((dt/dx)_{b}\) is the slope of the void volume marker, and \((dt/dx)_{n}\) is the slope of the internal volume marker. The molecular partition radius of the solute may be calculated using the following expression

\[
A = a_{0} + b_{0} \frac{f}{c},
\]

Here \(a_{0}\) and \(b_{0}\) are calibration constants for a set of particles of given radii in a given gel and are determined independently (30-33). The markers used were tobacco mosaic virus (void volume marker) in water (internal volume marker), M3-2 virus \((r = 130 \, \text{A}, \text{Miles Laboratories})\), thyroglobulin, ferritin, and aldolase \((r = 85, 61, \text{and} 48 \, \text{A}, \text{respectively, Pharmacia})\).

**Stopped Flow Spectrometry**—The pseudo-first order rate constants for the fast reaction between LDL and sodium deoxycholate at 25 \(^{\circ}\)C were determined using a Durrum model D-110 stopped flow spectrometer. The mixing volume was 0.30 ml (0.15 ml/syringe). The dead time for mixing was 4 ms in a 2-cm cuvette at 60 p.s.i.

In all the experiments described herein, each record consisted of a total of 200 data points, collected at equal time intervals over a total collection time of 1.0 s at 484 nm. After 2 s, the fast process was essentially completed. Usually 10 or more records were stored and averaged for each data set and the base-line was subtracted. The pseudo-first order rate constant \(k'\) was then calculated for each of the first 25 data points and averaged.

The set of reactions may be written as

\[
A + B \rightarrow AB \rightarrow C + D
\]

where \(A\) and \(B\) are LDL and sodium deoxycholate, \(AB\) is the LDL-sodium deoxycholate complex, \(C\) and \(D\) represent all protein-lipid complexes and lipid-detergent complexes, respectively, and \(k_{1}\) and \(k_{-1}\) are the bimolecular and first order rate constants. The first order rate constants for lipid displacement are represented by one average constant, \(k_{1}\). The change in free sodium deoxycholate concentration may be described as

\[
\frac{d[B]}{dt} = k_{1}[A][B] - k_{-1}[AB] - k_{1}[AB]
\]

Since \(k_{1}\) is very small with respect to \(k_{1}\) and \(k_{-1}\) (see Fig. 3)

\[
\frac{d[AB]}{dt} = k_{1}[A][B] - k_{-1}[AB]
\]

The ratio of unreacted LDL at time 0 to that at time \(t\), \(A_{t}/A_{0}\), may be represented in terms of the LDL carotenoid absorbance as \(A_{t}/A_{0} = (Abs_{-} - Abs_{t})/(Abs_{0} - Abs_{t}) = (Abs_{0}/Abs_{t})\). If conditions are chosen such that the reaction under investigation is pseudo-first order with respect to \(A\) (LDL), the pseudo-first order rate constant may be taken as

\[
k' = \frac{1}{t} \ln(\Delta Abs_{0}/\Delta Abs_{t})
\]

This expression was used to evaluate the pseudo-first order rate constant in several stopped flow experiments in which the LDL concentration was held constant and the sodium deoxycholate concentration was varied.

**Visible Absorption Spectroscopy**—The slow process for formation of the LDL-sodium deoxycholate mixed micelles

\[
AB \rightarrow C + D
\]

at 25 \(^{\circ}\)C was followed at 484 nm using a Gilford model 222 spectrophotometer. Low density lipoprotein in 8.5 buffer was used as a blank. This sample had a 0.275 at 484 nm using a buffer blank with a concentration of 4.81 mg/ml. To two other aliquots of this same LDL, solid sodium deoxycholate was added to final concentrations of 10 and 20 mM, respectively. The difference in absorbance between the two samples and the blank was monitored as a function of time up to 77 h and plotted.

**RESULTS**

**Scanning Molecular Sieve Chromatography**—Representative difference profiles of LDL in 9.7 buffer and 10 mM sodium deoxycholate-9.7 buffer on Bio-Gel A-5m at 220 nm are illustrated in Fig. 2. The time-dependent centroid movements of varying concentrations of sodium deoxycholate, LDL in 9.7 buffer, and LDL in varying concentrations of sodium deoxycholate-9.7 buffer were used to calculate the partition radii of these solutes, based on the centroid movements of four column calibration markers as previously described (Table I).

A plot of sodium deoxycholate radius \(\kappa_{1}\) versus concentration was used to estimate the upper limit of the CMC of sodium deoxycholate as 2.5 mM. However, although the micelle transition is fairly sharp, no data points were taken between 1.0 and 2.5 mM sodium deoxycholate, indicating that the viscosity value was 1.5 mm in this buffer (see Appendix). Also, an upper limit value of 21 + 0.5 A was found for the stable sodium deoxycholate micelle in this buffer.

Table I indicates a sodium deoxycholate and LDL concentration dependence on the processes of sodium deoxycholate binding and lipid displacement. Although the compositions of the particles produced in these experiments could not be determined, it is not unreasonable to assume LDL particles in the presence of 1 mM DOC (below CMC) are intact, with approximately a 27% increase in radius due to the binding of sodium deoxycholate monomers.

Furthermore, in LDL-sodium deoxycholate solutions in which the sodium deoxycholate concentration was 2.5 mM or greater (above CMC), a heterogeneous mixture of complexes was produced which may have contained varying amounts of sodium deoxycholate and lipid. It is also apparent from Table I that, in micellar solutions, increasing the sodium deoxycholate to LDL weight ratio within each set results in a decrease in the weight average partition radius, with the smallest complex produced having a radius of 25 A, approaching that of the sodium deoxycholate micelle itself.

Examination of Fig. 2b reveals two peaks, one a sharp, slower moving peak and the other a broad, fast moving peak. The sharp peak is assumed to correspond to apo-B-sodium deoxycholate or apo-B-lipid-sodium deoxycholate complexes, depending on the sodium deoxycholate to LDL ratio. The broad peak moves at the same rate in both cases as the LDL, 1 mM sodium deoxycholate complex, indicating that this peak may represent swollen LDL-sodium deoxycholate complexes. However, this peak is much broader than that of the LDL, 1
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A lipid displacement was studied further using the higher concentration of 20 mM sodium deoxycholate in 8.5 buffer. These data are summarized in Tables II and III. In addition to the LDL concentration dependence on particle size, these data indicate a time dependence as well. At high LDL concentrations, the particle size increased in the presence of 20 mM sodium deoxycholate after 3 h at room temperature, consistent with the phenomenon previously observed for solutions in which the sodium deoxycholate/LDL ratio was low. However, at lower LDL concentrations, particle size decreased rapidly and two peaks were observed, even after only 30 min of incubation in 20 mM sodium deoxycholate. Apparently, both particles, or populations of particles, represented by the two peaks contained lipid, since carotenoid was present as evidenced by the absorbance at 484 nm (Table II).

The particle classes having partition radii of 46–53 Å and

![Table I](image)

**Table I**

**Partition radius versus concentration of LDL in 8.7 buffer plus varying concentrations of sodium deoxycholate**

The radii were calculated based on a linear fit of r versus φ of MS-2 virus, thyroglobulin, ferritin, and catalase using Equation 8, where φ_0 = 11.83 and b_0 = 104.85. Data shown for LDL in buffer alone represent the average of two determinations. The standard deviation in the fitted radii is 3.25 Å in each case (Bio-Gel A-5m).

<table>
<thead>
<tr>
<th>[Sodium deoxycholate]</th>
<th>[apo-LDL]</th>
<th>Radius</th>
<th>φ</th>
<th>M^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>µg/ml</td>
<td>Å</td>
<td></td>
<td>x 10^-3</td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>98.85</td>
<td>0.967</td>
<td>2.68</td>
</tr>
<tr>
<td>50</td>
<td>99.90</td>
<td>2.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>161.99</td>
<td>2.81</td>
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<tr>
<td>200</td>
<td>206.19</td>
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<td>25</td>
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<td>100</td>
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<td>200</td>
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<td>2.5</td>
<td>11.93</td>
<td>79.98</td>
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<td>100</td>
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<td>200</td>
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<td>5.0</td>
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<td>200</td>
<td>78.93</td>
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<td>7.5</td>
<td>20.21</td>
<td>26.51</td>
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<tr>
<td>200</td>
<td>54.81</td>
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</table>

* M^* = 4πN(radius × 10^-3)/3, assumes spherical particle.

![Table II](image)

**Table II**

**Partition radius versus concentration of LDL in 8.5 buffer and 20 mM sodium deoxycholate-8.5 buffer**

The radii were calculated based on a linear fit of r versus φ of thyroglobulin, ferritin, and catalase using Equation 8, at 484 nm, where φ_0 = 19.89 and b_0 = 76.54. The standard deviation in the fitted radii in each case is 5.89 Å (Bio-Gel A-1.5m).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>[apo-LDL]</th>
<th>Incubation time^*</th>
<th>Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 8.5 buffer</td>
<td>1.72</td>
<td></td>
<td>97.96</td>
</tr>
<tr>
<td>20 mM sodium deoxycholate-8.5 buffer</td>
<td>1.72</td>
<td>30 min</td>
<td>97.19</td>
</tr>
<tr>
<td>8.5 buffer</td>
<td>1.72</td>
<td>3 h</td>
<td>107.14</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>1 h</td>
<td>55.86</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>4 h</td>
<td>52.80</td>
</tr>
</tbody>
</table>

^* All samples were incubated at room temperature.
Table III

Partition radius versus concentration of LDL in 8.5 buffer and 20 mM sodium deoxycholate-8.5 buffer.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>[apo-LDL] mg/ml</th>
<th>Incubation time</th>
<th>Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 8.5 buffer</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.95</td>
<td>86.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.48</td>
<td>87.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>88.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mM sodium deoxycholate-8.5 buffer</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>28.31*</td>
<td></td>
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</tr>
<tr>
<td>0.95</td>
<td>93.37</td>
<td></td>
<td></td>
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<tr>
<td>0.48</td>
<td>83.42</td>
<td></td>
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</tr>
<tr>
<td>0.24</td>
<td>47.44</td>
<td></td>
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</tr>
<tr>
<td>0.24</td>
<td>45.91</td>
<td></td>
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<tr>
<td>57.39</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>65.05</td>
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</tbody>
</table>

* All samples were incubated at room temperature.

The radii were calculated based on a linear fit of the r versus a of thyroglobulin, ferritin, and catalase using Equation 8 at 280 nm, where a0 = 19.89 and b0 = 76.54. The standard deviation in the fitted radii is 5.89 Å in each case (Bio-Gel A-1.5m).

75–65 Å remained relatively constant in size and shape (Tables II and III).

It is possible that the particle having a partition radius of approximately 100 Å and changing in both size and amount may represent an apo-B-lipid-sodium deoxycholate complex in which the protein unfolds as more lipid is removed with time. Such a process would result in an increase in the partition radius of apo-B-lipid-sodium deoxycholate, as well as an increase in the amount of this complex produced with time. The sum of our data supports this assumption, as discussed in detail in a subsequent paper (41).

Hence, the particle class which remained relatively constant may represent either a smaller apo-B-lipid-sodium deoxycholate complex which is relatively stable or a protein-free lipid-sodium deoxycholate complex. However, since the amount of this complex did not increase with time, as would be expected for lipid-sodium deoxycholate complexes, the former case is more likely. That is, the two peaks may represent two different populations of apo-B-lipid-sodium deoxycholate complexes in which the physical state of the protein is altered through differential binding of sodium deoxycholate and lipid (Fig. 2b).

Stopped Flow Kinetics—The rate of the initial event, formation of the swollen LDL-sodium deoxycholate complex, was measured by following the increase in absorbance of LDLCarotenoid at 484 nm at 25 °C using a stopped flow spectrometer. This process is a second order reaction in which the change in the absorbance of carotenoid is directly proportional to the binding of sodium deoxycholate or the change in free sodium deoxycholate concentration. However, since the molar ratio of sodium deoxycholate to LDL was approximately 1,000–25,000/liter in all experiments, the reaction would be assumed to be pseudo-first order with respect to LDL. Results are given in Fig. 3.

Rate constants for nonincubated LDL samples were the same within experimental error as those for LDL incubated for 20 h in 1 mM sodium deoxycholate. Both sets were fitted to the linear function $k_{obs} = 0.6720 \pm (0.5386) \text{mM sodium deoxycholate over the range } 0.5–20 \text{ mM sodium deoxycholate.}$

The degree of error in the fit prohibits accurate assessment of the true first order rate constant from the intercept, while the slope yields a bimolecular rate constant of approximately 539 ± 52 M−1 s−1. Although the equilibrium constant may not be accurately calculated from the ratio of slope to intercept, it is undoubtedly greater than 1, indicating that the binding of sodium deoxycholate to LDL is a spontaneous process. Furthermore, the fact that the rate constants for nonincubated LDL are not statistically different from those for 1 mM sodium deoxycholate-incubated LDL suggests that binding of sodium deoxycholate monomers below CMC is not cooperative. Also, the presence of 1 mM sodium deoxycholate appears to have even less effect at high sodium deoxycholate concentrations when micelles are present.

Preferential Binding of Sodium Deoxycholate—The ln C versus X^2 plot of LDL in 8.5 buffer revealed a very small degree of curvature, indicating that there is very little concentration-dependent self-association of LDL particles at 20 °C.

The whole cell weight average molecular weight calculated from this plot was found to be 2.56 ± 0.15 × 10^6 g/mol (Table IV), which is consistent with published values (3–5). Using $v_s = 0.967 \text{ ml/g (2)}$, the radius of an equivalent sphere of that weight would be 98.6 ± 0.6 Å, agreeing very closely with SMSC data. The ratio of these radii is equivalent to the frictional ratio and was used to calculate the axial ratio for a prolate ellipsoid as 1.67 (Table V).

The ln C versus X^2 plot of LDL in 1 mM sodium deoxycholate-8.5 buffer at 20 °C again indicated that there is very little concentration-dependent self-association taking place. The small degree of curvature in this plot and that for LDL allowed for accurate fitting of these data to straight lines, the slopes of which (shown in Table IV) were used in Equation 4 to estimate $e_i$ of the LDL, 1 mM sodium deoxycholate complex as 0.04 g of sodium deoxycholate bound/g of LDL, resulting in a $q = 0.945 \pm 0.002 \text{ ml/g and a preferential interaction term of } e_i = +0.034$, indicating preferential binding of sodium deoxycholate. The molecular weight was re-evaluated to be 2.67 ± 0.01 × 10^6 g/mol. Using $q = 0.945 \text{ ml/g}$ with this whole cell weight average molecular weight gave the radius of an equivalent sphere as 99.96 ± 0.18 Å (Table IV). Comparison of this radius with that from SMSC indicated an axial ratio of 5.56 for a prolate ellipsoid (Table V). Although these radii were determined in two different buffer systems, in which the effects on sodium deoxycholate micelles are apparent, it is assumed that the radius of the LDL, 1 mM sodium deoxycholate complex would change very little from 9.7 to 8.5 buffer, since only sodium deoxycholate monomers are present.

Visible Absorption Spectroscopy—The change in absorbance of LDL in 1 mM sodium deoxycholate at 25 °C was monitored at 683 nm using a stopped flow spectrometer. This process is a second order reaction in which the change in the absorbance of carotenoid is directly proportional to the binding of sodium deoxycholate or the change in free sodium deoxycholate concentration. However, since the molar ratio of sodium deoxycholate to LDL was approximately 1,000–25,000/liter in all experiments, the reaction would be assumed to be pseudo-first order with respect to LDL. Results are given in Fig. 3.

FIG. 3. Pseudo-first order rate constants for LDL-sodium deoxycholate interaction versus concentration of sodium deoxycholate after mixing. The values of pseudo-first order rate constant $k'$ were calculated as described under "Materials and Methods" at 25 °C and are given in s−1, while sodium deoxycholate concentration is given in millimolarity. ○, LDL in sodium deoxycholate incubated for 20 h prior to the experiment; □, nonincubated. The theoretical values represented by --- are based on the linear function $k_{obs} = (0.6720 ± 0.7007) + (0.5386 ± 0.0629) \text{ mM sodium deoxycholate.}$ The global goodness of fit, $R^2$, to this model was found to be 0.958.
**TABLE IV**

**Molecular sizes of LDL and LDL, 1 mM sodium deoxycholate complexes**

The whole cell weight average molecular weights, as well as the effective specific volume of LDL, 1 mM sodium deoxycholate, were determined from the sedimentation equilibrium runs in either 8.5 mM buffer or 1 mM sodium deoxycholate-8.5 buffer, in the presence of 0.01% EDTA, 0.01% Thimerosal, and 0.002% phenylmethylsulfonyl fluoride, at 5227 rpm at 20 °C. The radius is that of an equivalent sphere, calculated from the whole cell weight average molecular weight. The concentration of LDL was approximately 0.8 mg/ml in each case.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( d \ln C/\delta x^2 )</th>
<th>( \bar{c}_n )</th>
<th>( \bar{a}_x )</th>
<th>( \bar{u} )</th>
<th>Whole cell ( M_w )</th>
<th>Radius ( \bar{A} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>0.60712</td>
<td>0.967</td>
<td>0.84</td>
<td>0.034</td>
<td>2,560,185 ± 49,866</td>
<td>98.60 ± 0.64</td>
</tr>
<tr>
<td>LDL, 1 mM sodium deoxycholate</td>
<td>0.75022</td>
<td>0.945 ± 0.002</td>
<td>0.04</td>
<td>0.034</td>
<td>2,666,822 ± 13,893</td>
<td>99.96 ± 0.18</td>
</tr>
</tbody>
</table>

* The partial specific volume of LDL was taken from Ref. 2. That for LDL, 1 mM sodium deoxycholate was calculated from Equation 5.
* The binding was calculated from Equation 4, using \( \bar{c}_n = 0.779 \) ml/g.
* The preferential interaction term was calculated from Equation 6.

**TABLE V**

**Molecular size and shape of LDL and LDL, 1 mM sodium deoxycholate complexes**

Radii were determined by SMSC in 9.7 buffer at 25 °C and molecular weights were determined by analytical ultracentrifugation at 20 °C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( M_w )</th>
<th>Radius ( \bar{A} )</th>
<th>( r_{eq} )</th>
<th>Approximate shape</th>
<th>a/b</th>
</tr>
</thead>
</table>
| LDL                           | 2.560 ± 0.150 | 100.98' | 1.024 | Prolate ellipsoid 1.67
| LDL, 1 mM sodium deoxycholate  | 2.667 ± 0.014 | 128.56' | 1.286 | Prolate ellipsoid 5.56 |

* The frictional ratio, where \( r_{eq} \) is the radius of an equivalent sphere calculated from \( M_w \), using Equation 9.
* The axial ratio, calculated from Equation 10 (prolate ellipsoid).
* Averaged value from Table I and II.
* The radius was determined in 9.7 buffer and the \( M_w \) was determined in 8.5 buffer.

The frictional ratio, where \( r_{eq} \) is the radius of an equivalent sphere calculated from \( M_w \), using Equation 9. The axial ratio, calculated from Equation 10 (prolate ellipsoid). Averaged value from Table I and II. The radius was determined in 9.7 buffer and the \( M_w \) was determined in 8.5 buffer.

**Fig. 4. Change in absorbance of LDL in the presence of micellar sodium deoxycholate.** Absorbance of LDL in the presence of 10 mM (A) and 20 mM (B) sodium deoxycholate in pH 8.5 buffer was followed at 484 nm at 25 °C up to 77 h (inset, 24–77 h) using LDL in pH 8.5 buffer as a blank. The concentration of LDL in both samples and the blank was 4.83 mg/ml. The initial absorbance changes were due to solubilization of carotenoids by sodium deoxycholate. However, after 4 h, a significant contribution to these changes in absorbance was made by light scattering due to the appearance of turbidity in the samples. These light-scattering effects could not be distinguished from the absorbance changes due to carotenoid solubilization, and were presumed responsible for the continued linear increases observed.

Heating the LDL in the presence of SDS and mercaptoethanol to 75 °C for 2 h gave five major bands, most of which were similar to minor bands in control samples, with the exception of one prominent band which had a reduced mobility from the major band in control samples. Three very minor bands also appeared. Such changes upon heat treatment have been observed previously (7) and indicate the presence of either several subunits in LDL or one subunit with labile protein domains. In any case, several or all of the resultant peptides apparently have the ability to reassociate or aggregate to an appreciable extent.

Samples of LDL in the presence of sodium deoxycholate gave essentially the same results, regardless of sodium deoxycholate concentration (Fig. 56). The mobility of the major band in these preparations was decreased slightly from that in the absence of sodium deoxycholate. Such increases in particle size are consistent with SMSC results.

After a 10-day incubation under nitrogen at 4 °C in the presence of 1 mM sodium deoxycholate, LDL gave two major bands and one prominent minor band. Similar incubations in...
the presence of 0.2 mM sodium deoxycholate gave one major band and two prominent minor bands. Although the overall appearance of these gels was not very different from that of the LDL control, these results suggest that in the presence of sodium deoxycholate an association or elongation may take place involving the largest and smallest peptides.

**DISCUSSION**

**Characterization of Sodium Deoxycholate**—The CMC of sodium deoxycholate was determined as 1.3 and 1.5 mM in 9.7 and 8.5 buffer, respectively, by viscometry at 25 °C (see Appendix). These values are in agreement with previously reported values at moderately high ionic strength (24, 25). The radii of sodium deoxycholate micelles in these buffers were determined by SMSC to be 21 (9.7 mM) and 28 Å (8.5 mM). However, these micelles are not spherical, but are assumed to be elongated complexes of relatively low aggregation number formed by a continuous association of monomers (mass action model of micellization) (35). The sodium deoxycholate anion has been shown by ellipsometry to have hydrophilic one outwards" (36). Such an aggregation mechanism would result in an increase in micelle radius dependent on the shorter dimension (3.1 Å) of the sodium deoxycholate monomer. Therefore, using the micelle radii above, the aggregation numbers and axial ratios of these micelles would be 14 and 3.1, respectively, for 9.7 buffer and 18 and 4.5, respectively, for 8.5 buffer.

**Sodium Deoxycholate Binding and Lipid Displacement Reaction Dynamics**—Overall, the SMSC results show that increasing the sodium deoxycholate to LDL weight ratio appears to affect at least three processes. The first process is the saturation of the LDL particle with sodium deoxycholate monomers, giving a swollen LDL-sodium deoxycholate complex. The second process appears to involve a further "cooperative" sodium deoxycholate binding and lipid displacement in which the protein is unfolded to increase the surface area for sodium deoxycholate interaction as lipid is removed. Both processes would result in an increase in the partition radius and would be indistinguishable by that criterion alone. A third process, which is time dependent, appears to have the opposite effect on particle radius. That is, with increasing incubation time, the particle size decreases. The mechanism by which this process takes place is unclear, since it is not due merely to removal of lipid but apparently involves a change in the physical state of the apoprotein as well, i.e., one in which the protein is dispersed into smaller subunits by sodium deoxycholate micelles or one in which the protein undergoes a change to a more compact (globular) conformation.

All hydrodynamic data for LDL in the absence of sodium deoxycholate presented in Tables I-V are consistent with previously reported values (1, 3, 37-41). In the presence of 1 mM sodium deoxycholate (below CMC), the average radius increased by 27% (102 to 129 Å). However, the molecular weight of this complex was only 2.67 × 10^8, or an increase of only 5.1% over the molecular weight of LDL alone (2.56 × 10^8). These results suggest that the LDL, 1 mM sodium deoxycholate complex is very asymmetrical with an axial ratio of approximately 5.6, assuming a prolate ellipsoid. Additionally, the amount of sodium deoxycholate bound in this complex was only 0.04 g/g of LDL, or approximately 250 molecules of sodium deoxycholate/LDL particle.

Assuming that the sodium deoxycholate monomers interact with each other to a minimal extent at this concentration and considering that the surface area of LDL is very large (~1.30 × 10^5 Å^2), and further assuming that the 250 or so monomers are evenly distributed over the surface, the increase in surface area of 84 Å^2/monomer upon binding of sodium deoxycholate would result in a complex radius of only 110 Å. Since the
radius of the LDL, 1 mM sodium deoxycholate complex was consistently found to be much larger, the sodium deoxycholate monomers cannot be randomly distributed over the LDL surface, but apparently bind to specific regions (presumably apoprotein). If the sodium deoxycholate does indeed bind to apo-B preferentially, this translates to an initial binding ratio of 0.17-0.62 g of sodium deoxycholate/g of apo-B in the LDL, 1 mM sodium deoxycholate complex, which is only one-third to one-half of the amount bound to delipidated apo-B at high sodium deoxycholate concentrations (12).

In studying the binding of SDS to apo-B, Steele and Reynolds (7) showed that the conformation of delipidated apo-B in monomeric solutions of SDS was not significantly different from that in intact LDL. It is likewise assumed that the conformation of apo-B in the LDL, 1 mM sodium deoxycholate complex is not altered significantly, but that there is a maximum interaction with sodium deoxycholate monomers resulting in a “distortion” of the LDL surface and a large increase in particle radius. Although the precise arrangement of the protein, sodium deoxycholate, and lipid in such complexes is unknown, it is not unlikely that a good deal of this distortion is produced by the pulling of apo-B outward, displacing it slightly from its normal position in the LDL particle as sodium deoxycholate monomers bind. However, the possibility that this initial increase in radius is due to sodium deoxycholate binding to and distortion of specific lipids on the LDL surface cannot be ruled out.

Increasing the concentration of sodium deoxycholate above the CMC resulted in a decrease in the partition radius of LDL which was proportional to the sodium deoxycholate to LDL ratio. Such particles presumably represent a heterogeneous mixture of complexes containing varying amounts of protein, sodium deoxycholate, and lipid. In solutions of 5 or 10 mM sodium deoxycholate-9.7 buffer, changes in particle radius were most notable and two peaks were observed, one a sharp, slow moving peak and the other a broad, fast moving peak (Fig. 2B). The broad peak moved at the same rate as that of the LDL, 1 mM sodium deoxycholate complex but was much broader, indicating more heterogeneity, possibly as a result of varying degrees of binding of sodium deoxycholate monomers to intact LDL particles, or the presence of delipidated apo-B-sodium deoxycholate complexes, the lipid contents and radii of which are inversely related to the sodium deoxycholate to LDL ratio, as discussed in a subsequent paper (41).

The particle size distribution was also observed to be dependent on the incubation time in the presence of 20 mM sodium deoxycholate-8.5 buffer. The data presented in Tables II and III are for two independent preparations of LDL. The differences in radii of the several preparations in the absence of sodium deoxycholate are not uncommon (38-40). However, the percentage changes in radii for the two preparations in the presence of sodium deoxycholate were very similar, with differences in the time required to affect such changes attributed to differences in the sodium deoxycholate/LDL ratios. In general, low sodium deoxycholate/LDL ratios resulted in initial increases in particle radii, while higher ratios resulted in the appearance of two separate particle populations with smaller radii in each case. The incubation studies indicate a precursor-product relationship between the larger particles and the two smaller populations.

The two peaks apparently represent apo-B-lipid-sodium deoxycholate complexes which differ in their lipid compositions and possibly in the physical states of their apoprotein. The fact that these complexes still contained a large amount of lipid was evidenced by their relatively large sizes (46-65 Å), as well as the fact that they continued to absorb at 484 nm, although some of this may have been due to light scattering as a result of turbidity which appeared in the samples incubated for longer than 1 h. However, it has previously been shown that large excesses of detergent are needed to completely delipidate apo-B (7, 15, 41). Such excesses were not present in studies using 20 mM sodium deoxycholate-8.5 buffer, as the largest sodium deoxycholate/apo-B ratio was only 34.5:1. However, in studies using 5 and 10 mM sodium deoxycholate-9.7 buffer, sodium deoxycholate/apo-B ratios ranging from 41.5-166:8:1 resulted in particles ranging from 37-25 Å, respectively, indicating that some of these complexes may have been delipidated.

Taken collectively, the SMSC results for LDL suggest a mechanism for sodium deoxycholate binding and lipid displacement. The initial increase in the radius of LDL in the presence of sodium deoxycholate is apparently the result of the binding of sodium deoxycholate monomers and distortion of the LDL particle. This increase occurs at any sodium deoxycholate concentration, but above CMC it is offset by a slow decrease in particle radius resulting from lipid displacement or possibly even scission of the original LDL particle into two separate protein-lipid-sodium deoxycholate complexes of differing compositions when the sodium deoxycholate/apo-B ratio is low. When the sodium deoxycholate/apo-B ratio is high (41.5:1), an initial increase in radius is also seen, but the complexes are more heterogeneous. This is apparently due to the additional presence of apo-B-sodium deoxycholate complexes in which the protein is unfolded or elongated as lipid is removed (41). If the ratio is increased, these large complexes can be seen to give rise to smaller complexes as a result of dissociation. This interpretation is also supported by additional SMSC data for apo-B-sodium deoxycholate complexes, as well as by results of analytical ultracentrifugation, compositional analysis, and SDS-PAGE (41).

Additional support for the hypothesis of differences in the rates of sodium deoxycholate binding and lipid displacement was provided by stopped flow and visible absorption spectroscopy. The binding of sodium deoxycholate to LDL is relatively fast (Fig. 3) compared to lipid displacement and solubilization (Fig. 4). Furthermore, the rate of binding appears to be linearly dependent on the sodium deoxycholate concentration, with preincubation in 1 mM sodium deoxycholate having a minimal effect. This initial interaction has a bimolecular rate constant of ~539 M⁻¹ s⁻¹, which although not large compared to enzyme-substrate reactions (10⁻¹⁰ M⁻¹ s⁻¹), is certainly faster than those processes observed by visible absorption spectroscopy (Fig. 4). Additionally, the linear nature of the rate versus (sodium deoxycholate) plot (Fig. 3) suggests that the initial interaction is not cooperative even though the aggregation number of sodium deoxycholate changes throughout this range. This is in contrast to the very slow processes observed by visible absorption spectroscopy, especially in the presence of 20 mM sodium deoxycholate. The change in absorbance at 484 nm of LDL in 20 mM sodium deoxycholate exhibited a slight albeit significant sigmoidality during the first 7 h, suggesting cooperative increments in the binding of sodium deoxycholate and displacement and solubilization of protein and lipid by sodium deoxycholate micelles. The linear increase in turbidity at later times was apparently the result of the production of a heterogeneous mixture of apo-B-lipid-sodium deoxycholate and lipid-sodium deoxycholate complexes, some of which became insoluble due to the limited amount of sodium deoxycholate available under the conditions of a low sodium deoxycholate/apo-B ratio. In solutions where the sodium deoxycholate/apo-B ratio was high (Table
Therefore, in summation, we may conclude that: 1) the binding of sodium deoxycholate to LDL is a relatively fast process, with a bimolecular rate constant of $-539 \pm 63 \text{ M}^{-1} \text{s}^{-1}$. 2) The initial 27% increase in the radius of LDL upon binding of sodium deoxycholate is apparently due to a sodium deoxycholate-induced elongation or distortion of certain portions of the LDL particle, since the relatively small amount of sodium deoxycholate bound (0.04 g/g of LDL) can only account for a 6-7% increase at best. This suggests that the binding of sodium deoxycholate to LDL is a highly specific process. 3) The processes of lipid displacement and solubilization of apoprotein from LDL by sodium deoxycholate are greatly different. 4) The efficiency of lipid displacement and solubilization from LDL by sodium deoxycholate is proportional to the sodium deoxycholate/LDL ratio.

Acknowledgments—The suggestions and advice of Drs. Waldo R. Fisher and Bruce W. Patterson on the isolation apo-B are greatly appreciated.

REFERENCES

Additional references are found on p. 3654.
The critical micelle concentration (CMC) of various ionic and non-ionic surfactants was determined from spectrofluorimetry in aqueous suspension (1, 2). CMC is based on the spectroscopic differences in the presence of surfactants above and below the critical micelle concentration. The critical micelle concentration of the various ionic surfactants was also evaluated based on the normal red shift with the hydrophobic side pointing inwards and viscosity, where \(r = 5.186 \times 10^{-10} \text{m}^2\text{kg}^{-1}\text{s}^{-1}\). The critical micelle concentration was measured using spectrophotometry (1, 2). The CMC and normal red shift differences were also used to determine the CMC of various surfactants.

The critical micelle concentration of sodium deoxycholate was determined from the absorption spectra of phospha-ester chloride in de-oxycholate (DC).

**Absorption spectra of phospha-ester chloride in de-oxycholate**

![Absorption spectra of phospha-ester chloride in de-oxycholate](image)

The absorption spectra of phospha-ester chloride in de-oxycholate were determined at 480-493 nm in a bath of liquid nitrogen. The critical micelle concentration of sodium deoxycholate was determined from the minima in the flow time, as illustrated in Figure 2.

**Table 1**

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Ionic strength</th>
<th>CMC (mM)</th>
<th>CMC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium deoxycholate</td>
<td>0.104</td>
<td>0.092</td>
<td>0.467</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate</td>
<td>0.095</td>
<td>0.095</td>
<td>0.095</td>
</tr>
<tr>
<td>Sodium methyl sulfate</td>
<td>0.095</td>
<td>0.095</td>
<td>0.095</td>
</tr>
<tr>
<td>Sodium trimethyl ammonium bromide</td>
<td>0.095</td>
<td>0.095</td>
<td>0.095</td>
</tr>
</tbody>
</table>

The critical micelle concentration of sodium deoxycholate is 0.7 and 0.5 mM after it was also determined by viscosimetry to be 0.3 and 0.15 mM respectively, as indicated in Figure 2. At an ionic strength of 0.2, the value of the CMC is constant with values determined by the CMC method.

References

Human serum low density lipoprotein-sodium deoxycholate interaction.
J Q Oeswein and P W Chun


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