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Interactions of ionic and non-ionic surfactants with plasma low density lipoprotein

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Surfactants might be useful in facilitating the replacement of the interior of low density lipoprotein (LDL_2) (ρ = 1·02–1·063 g ml^{-1}) with drug molecules. We have used photon correlation spectroscopy, supported by sedimentation velocity measurements to study the effects of surfactants on LDL_2. Sodium dodecyl sulphate, cetrimide, and all non-ionic surfactants studied caused rapid increases of ca 50% in the Stokes' radius up to surfactant/LDL_2 molar ratios of ca 1000:1. This was interpreted as due to partial unfolding of the LDL_2 protein and intercalation of surfactant with the LDL_2 surface layer. At higher concentrations, ionic surfactants and non-ionics with HLB values <14·6 decreased the Stokes' radius due to delipidation of LDL_2. These interactions are similar to those between surfactants and biological membranes, thus LDL_2 might be a useful model system to study surfactant structure-activity relationships.

Plasma low density lipoprotein (LDL_2) with hydrated density 1·02–1·063 g ml^{-1} might be a suitable natural drug carrier especially for cytotoxic agents (Gal et al 1981; Mosley et al 1981). LDL_2 is also known to bind some drugs (Nilsen 1976; Lemaire & Tillement 1982) and this might alter their behaviour in-vivo. As part of a program aimed at understanding these interactions and eventually loading LDL_2 with drugs, we have studied the effects of ionic and a wide range of non-ionic surfactants on this lipoprotein by photon correlation spectroscopy (PCS) supported by analytical ultracentrifugation.

LDL_2 is a quasi-spherical particle of 10–12 nm radius and molecular weight 2–3 × 10^6 daltons (Laggner 1976; Kirchhausen et al 1980). It is about 80% lipid and 20% protein and comprises a structured lipid core (Deckelbaum et al 1977) of cholesterol esters (40% of LDL_2) and triglycerides (5%) surrounded by a monolayer of phospholipids (25%) and cholesterol (10%). The two chains of protein (apolipoprotein B, mol wt 250 000—Steele & Reynolds 1979) interact with about 20–30% of the phospholipid head groups (Yeagle et al 1977, 1978; Herak et al 1982) and are distributed over and within the particle so that 20–30% of the protein is exposed to solvent and readily available to proteolytic enzymes (Ikai 1975; Chapman et al 1978). Thus LDL_2 has some of the structural characteristics of membranes and so might be expected to interact with surfactants in a similar way.

This interaction entails the adsorption and penetration of surfactant monomers into the membrane leading to swelling, organizational changes, and permeability changes. Saturation of the membrane occurs at about 1–2 mole surfactant per mole of phospholipid. Addition of more detergent beyond this level causes breakdown of the membrane into lipid-surfactant-protein and lipid-surfactant mixed micelles. At higher surfactant concentrations, phospholipid is displaced from the lipid-surfactant-protein mixed micelles, whereas the size of the lipid-surfactant micelles decreases as the proportion of surfactant increases (Helenius & Simons 1975; Stubbs & Litman 1978).

LDL_2 is delipidated by high concentrations of anionic, cationic, and non-ionic surfactants (Helenius & Simons 1971). Zampighi et al (1980) have shown apolipoprotein B solublized in n-dodecyl octaethylene-glycol solutions to be rod shaped with dimensions 80 × 5 nm, whereas surfactant-phospholipid mixed micelles are typically spherical (radius 10 nm) at high surfactant concentrations (Helenius & Simons 1975). Thus LDL_2 should undergo dimensional changes in the presence of surfactants and these will be manifested as changes in

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its hydrodynamic properties. Ikai (1976, 1980) and Ikai & Hasegawa (1978) used sedimentation velocity measurements to study the effects of sodium dodecylsulphate, Tween 80, and Triton X-100 on LDL_2.

We have used photon correlation spectroscopy (PCS), previously used by De Blois et al (1973) to determine the effects of pH, ionic strength, and concentration on the Stokes' radius of LDL_2, to study the effects of sodium dodecyl sulphate, cetrimide, and several non-ionic surfactants on LDL_2. Up to molar ratios of ca 1000:1 (total surfactant/LDL_2) the surfactants caused virtually instantaneous increase in the Stokes' radius. This was probably due to partial unfolding of the apolipoprotein B, and intercalation of the surfactant with the surface layer of LDL_2 leading to particle assymetry. At higher molar ratios, corresponding to 1–2 moles surfactant per mole phospholipid, ionic surfactants and non-ionics with HLB values <14·6 caused rapid decreases in the Stokes' radius. However, polyoxyethylene 23 dodecyl ether (HLB 16·9) also caused a decrease but in 20 h. These decreases were interpreted as due to breakdown of LDL_2 into lipid-surfactant and protein-surfactant micelles.

MATERIALS AND METHODS

Lipoprotein. Human plasma was isolated from venous blood drawn from fasting normo-lipidaemic adults and the LDL_2 extracted immediately by rate zonal ultracentrifugation using a sodium bromide gradient (Patsch et al 1974). The sample was ultrafiltered (Diaflo Type XM-100A, Amicon Corp. Lexington, Mass, U.S.A.) to dilute the sodium bromide (1–10 000) and replace it with phosphate buffer (pH 7·4) made isotonic with sodium chloride and containing 0·01% EDTA. Samples were sterilized by filtration (0·22 μm) and aseptically packed under nitrogen, then stored below 8 °C and used within two weeks. The time from extraction to packing was 2–3 days, with all procedures being conducted at room temperature (ca 20 °C).

Surfactants. Brij 35 (polyoxyethylene 23 dodecyl ether, $C_{12}E_{23}$), Brij 58 ($C_{16}E_{20}$), Brij 78 ($C_{18}E_{20}$), and Myrj 52 (polyoxyethylene 40 stearate, $C_{18}E_{40}$) were supplied by Atlas Chemical Industries UK, Leatherhead, U.K. Brij 36T ($C_{12}E_{10}$) and Triton X-100 (polyoxyethylene 9–10 t-octylphenyl ether, $C_8PE_{9\cdot5}$) were obtained from Sigma London Chemical Company Ltd., Poole, U.K. Texofor A14 ($C_{16}E_{14}$) and Texofor A60 ($C_{16}E_{60}$) were supplied by ABM Chemicals, Stockport, U.K. Cetrimide (CTAB) was purchased from Evans Medical Ltd., Liverpool, U.K., and sodium dodecyl sulphate (SDS) was obtained from BDH, Poole, U.K.

Other reagents. Buffer salts were of analytical grade (BDH). Guanidine hydrochloride (practical grade) and subtilisin BPN′ (EC No. 3.3.21.14) Type VII were purchased from Sigma. All reagents were used as supplied. Aqueous solutions were made using glass distilled water.

Analysis. LDL_2 was quantified by refractive index measurements (refractive index increment $1\cdot71 \times 10^{-4}$ litres g^{-1}, Armstrong et al 1947) using a Rayleigh interference refractometer (Hilger and Watts, Type M154) with 1 cm cells and a white light source. Analyses were in agreement with those determined by the Lowry method (Lowry et al 1951) for apolipoprotein B (LDL_2 = 5 × protein concentration) using bovine serum albumin as the standard.

Photon correlation spectroscopy. A photon correlation spectrometer (Malvern Instruments, Model 4300) with 48 channels and later a Type 7027 with 60 channels was used with a He/Cd laser (Liconix) operating at 441·6 cm with a power of approximately 10 mW. All samples were thermostatted to 25 ± 0·1 °C and measurements were made at an angle of 90°.

Filtered (0·22 μm) surfactant solution (1–10% in isotonic phosphate buffer, pH 7·4) was added incrementally to filtered lipoprotein solution containing 5–10 mg ml^{-1} of LDL_2. Because of the tendency of LDL_2 to aggregate, solutions were gently mixed in the PCS cuvettes. If aggregation occurred, the solutions were refiltered (0·22 μm). Four determinations of the Stokes' radius were made starting 5 min after each addition.

In kinetic experiments the surfactant or guanidine hydrochloride solution was added at time zero and the changes in Stokes' radius followed by PCS. Experiments using digested LDL_2 were conducted by treating LDL_2 with subtilisin BPN′ (LDL_2:protease was 50:1 and 10:1 by weight) for 2 h at 25 °C then adding surfactant incrementally, and secondly, by digesting (LDL_2:protease = 50:1) surfactant-treated LDL_2 (molar ratio surfactant:LDL_2 was 800:1) and following the changes by PCS.

The second order cumulant method (Koppel 1972; Brown et al 1975) was used to estimate the frictional coefficient (f). The equivalent spherical radius (R) was then calculated using Stokes' Law ($f = 6.\pi.\eta.R$) where η is the viscosity (0·931 cP) of isotonic phosphate buffer (pH 7·4) at 25 °C. The cumulant method also gives an estimate of the polydispersity (NVD) of the particles in solution. Values of NVD <

0·1 are generally accepted as indicating monodispersity although this is an arbitrary demarcation.

Analytical ultracentrifugation. LDL_2 samples (1·4 mg ml^{-1}) were pretreated with surfactant $C_{12}E_{20}$ or $C_{12}E_{23}$ before loading into Beckman double sector cells fitted with aluminium filled epon centrepieces. Cells were housed in a Beckman AnF four place rotor and spun at 40 000 rev min^{-1} at 25 ± 0·5 °C in a Beckman L8-70 ultracentrifuge fitted with a Beckman Prep UV Scanner analysing at 280 nm. Each run included one native LDL_2 sample and two surfactant treated samples. Readings were taken every 10–15 min for 3 h, starting 30 min after surfactant treatment, and the apparent sedimentation coefficient calculated by standard methods (Chervenka 1973).

RESULTS

The Stokes' radius (R) of native LDL_2 from five donors was 10·9 to 13·4 nm in agreement with previous reports (De Blois et al 1973; Packard et al 1979). The samples were slightly polydisperse (NVD values 0·06–0·13). Considerable care was required in handling LDL_2, since agitation led to gross aggregation. When these aggregated samples of LDL_2 were refiltered (0·22 μm), the redetermined R and NVD values were no different from preaggregation values. No change in R or NVD occurred on dilution of LDL_2 with isotonic phosphate buffer in agreement with De Blois et al (1973) who reported an insignificant (0·15 nm) decrease on dilution.

Time dependent effects. There was no change in the radius of LDL_2 over the test period (Fig. 1a). $C_{12}E_{23}$ (Fig. 1a) and $C_{12}E_{10}$ (Fig. 1b) caused rapid (<2 min) increases in R which at high concentrations (>800:1) were followed by gradual decreases. This suggested that LDL_2 was being solubilized at these high concentrations. For $C_{12}E_{10}$ at 8000:1, the initial increase was slight indicating that solubilization occurred rapidly. This was not the case for $C_{12}E_{23}$ even at 30 000:1, but $C_{12}E_{23}$ at 8000:1 did reduce the radius to 14 nm over 20 h.

In 4 M guanidine hydrochloride solution an immediate increase to 15 nm was followed by a slow rise to 18 nm after 1·5 h. There was no decrease in R at later times.

Surfactant:LDL_2 versus R profiles. Incremental additions of surfactants caused increases in R up to 1000:1 to 2000:1 without significant changes in the NVD. Above 2000:1 there was a fall in R for LDL_2 treated with ionic surfactants or non-ionic surfactants with HLB values <14·6 ($C_{12}E_{10}$, $C_{16}E_{14}$, $C_8PE_{9.5}$) suggesting solubilization of LDL_2 (Fig. 2).

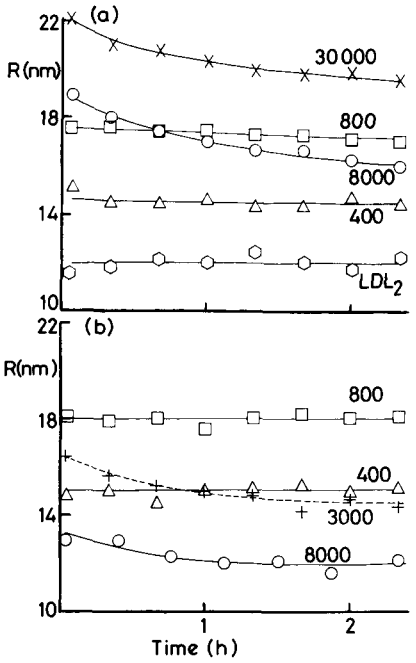

FIG. 1. Time dependent changes in the Stokes' radius (R) in the presence of (a) $C_{12}E_{23}$ and (b) $C_{12}E_{10}$ surfactants at various molar ratios. Numbers shown are the moles of surfactant per 2·5 × 10^6 g LDL_2.

These trends were reproducible across donors (Fig. 3) and within batches, but the variability of the response (pooled estimate s.d. = 1·1 nm) might have masked some small differences among surfactants.

Below 2000:1 aggregation was not a problem since the increases in R occurred without change in NVD. However, at high concentrations of $C_{12}E_{10}$ and $C_{16}E_{60}$, the NVD increased on some occasions suggesting aggregation was occurring. This is the most likely cause of the increases in R seen at high concentrations of these surfactants.

Zampighi et al (1980) reported that apolipoprotein B solubilized with $C_{12}E_8$ aggregated at pH 7·4 but not as much at pH 10. We found that the radius of LDL_2 at pH 10 exposed to increasing concentrations of $C_{12}E_{10}$ showed changes similar to those at pH 7·4 except the fall at high ratios continued to 13·8 nm at 8000:1 (cf Fig. 2c) and the NVD was smaller (0·13 cf 0·2). The value of 13·8 nm compares well with that for the 8000:1 data in Fig. 1b. Thus incremental additions of surfactant with mixing at high concentrations caused aggregation leading to the slight increases in radius at the highest concentrations of some surfactants (Fig. 2).

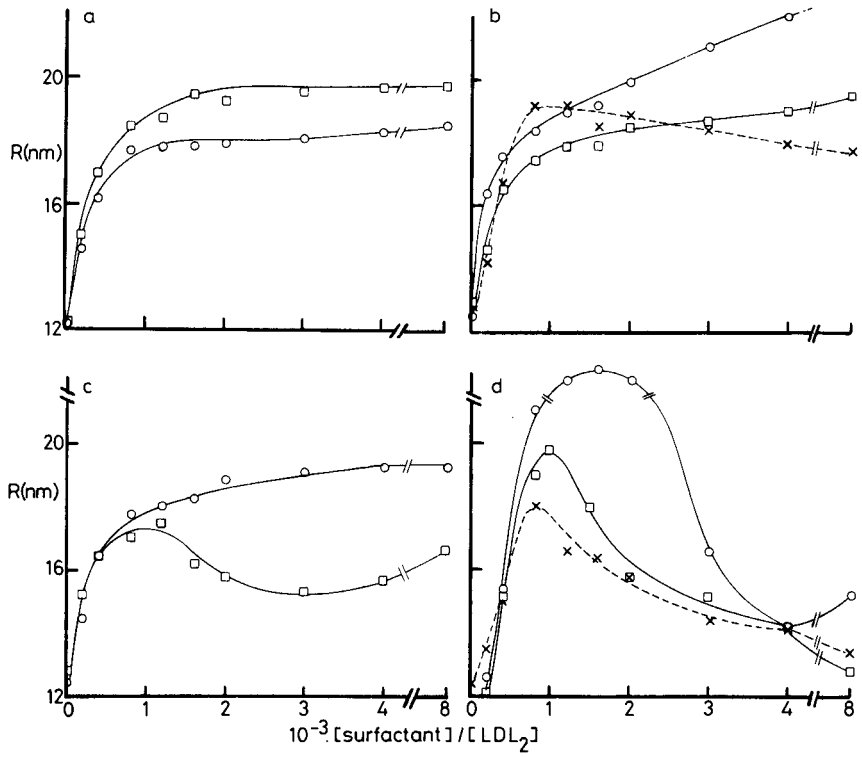

FIG. 2. Effect of surfactant concentration on the Stokes' radius (R) of LDL_2. Abscissa is the moles of surfactant per $2{\cdot}5 \times 10^6$ g LDL_2. (a) $C_{18}E_{40}$ (○), $C_{18}E_{20}$ (□); (b) $C_{16}E_{60}$ (○), $C_{16}E_{20}$ (□), $C_{16}E_{14}$ (×); (c) $C_{12}E_{23}$ (○), $C_{12}E_{10}$ (□); (d) SDS (○), CTAB (□), $C_8PE_{9\cdot5}$ (×). The Stokes' radius was determined 5 min after LDL_2 and surfactant were mixed.

Digested LDL_2. There were only minor increases (12·2 to 12·6 nm) in R on digestion with 2% and 10% subtilisin BPN'. The NVD was unchanged after digestion with 2% protease but there was an increase in NVD variability and magnitude with 10% subtilisin BPN'. Chapman et al (1978) have also reported a greater heterogeneity in particle size and a slightly larger mean diameter after tryptic digestion. Ikai & Yagisawa (1977) found a slightly decreased diameter after subtilisin BPN' treatment which they showed split the apolipoprotein B into five major fragments (70 000–160 000 daltons) after 30 min with 2% protease.

The increase in R on addition of $C_{12}E_{10}$ or $C_{12}E_{23}$ to 2% digested LDL_2 were significantly ($P < 0{\cdot}05$) less (2·2 nm at 800:1) than those for native LDL_2 (Fig. 4). With 10% digested LDL_2 the difference was 2·6 nm, or 50% less, at 800:1. These results were confirmed by digesting LDL_2 which had been pretreated with $C_{12}E_{23}$ at 800:1. This treatment caused a 2·5 nm fall (17·5 nm to 15 nm) in R over 20 min. R then remained unchanged even after addition of fresh subtilisin BPN'. Thus intact apolipoprotein B is required for about half of the overall increases observed in the radius of LDL_2.

Sedimentation velocity. The shape of the sedimentation profiles indicated that samples were slightly polydisperse. Average sedimentation coefficients

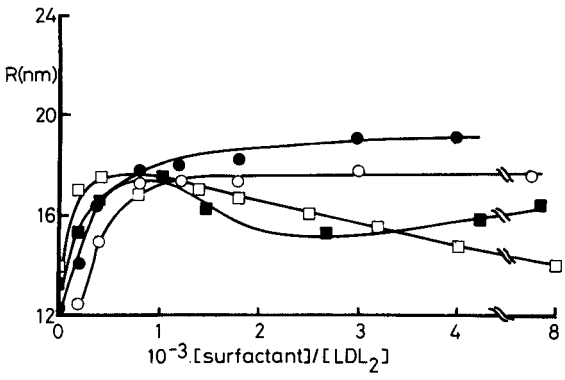

FIG. 3. Effect of $C_{12}E_{23}$ (○ ●) and $C_{12}E_{10}$ (□ ■) on the Stokes' radius of LDL_2 from various donors. ● IT, ○ TL, ■ GS, □ JS. Abscissa as in Fig. 2.

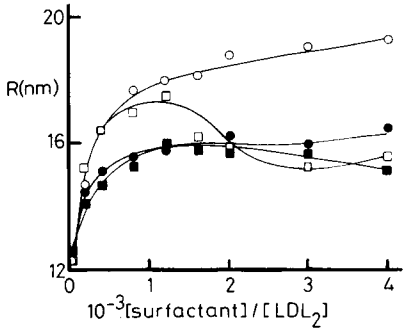

FIG. 4. Effect of $C_{12}E_{23}$ (circles) and $C_{12}E_{10}$ (squares) on the Stokes' radius of native LDL_2 (open symbols) and digested LDL_2 (filled symbols). Abscissa as in Fig. 2. LDL_2 was digested for 2 h at 25 °C with subtilisin BPN' before treatment with surfactant as in Fig. 2.

are shown in Table 1. The sedimentation coefficient (S) is given by (Tanford et al 1974):

$$S = \frac{M.(1 - \nu_L.\rho) + \delta(1 - \nu_S.\rho)}{f} \qquad (1)$$

where M is the molecular weight of LDL_2, ν_L and ν_s the partial specific volumes of LDL_2 and surfactant respectively, δ the grams of surfactant bound g^{-1} of LDL_2, ρ the solvent density, and f the frictional coefficient.

Table 1. Effect of $C_{12}E_{10}$ and $C_{12}E_{23}$ surfactants on the sedimentation coefficient (S) and Stokes' radius (R) of LDL_2.

Surfactant	Moles surfactant/ $2{\cdot}5 \times 10^6$ g LDL_2	S (10^{13}·s)	R (nm)
—	—	4·6	12
$C_{12}E_{10}$	800	3·7	17
	8000	7·0, −5	13
$C_{12}E_{23}$	800	5·7	17·5
	8000	4·8	19

Sedimentation velocity measurements at 40 000 rpm, 25 °C.

Assuming all the surfactant was bound, for $C_{12}E_{10}$ (ν_s = 0·958, Steele et al 1978) $S_{complex}/S_{LDL_2}$ equals 0·86 in reasonable agreement with the observed ratio of 0·8. So the decrease in S with $C_{12}E_{10}$ is consistent with an increase in f and hence R. For the more dense $C_{12}E_{23}$ (ν_x = 0·91, by interpolation from Steele et al 1978) $S_{complex}/S_{LDL_2}$ is 1·3 compared with 1·2 observed. So the small increase in S is interpreted as binding of more dense surfactant to increase S but this is in part compensated for by an increase in f.

At 8000:1 the $C_{12}E_{10}$ sample had obviously broken into a fast sedimenting component ($S = 7 \times 10^{-13}$ s) and a floating component ($S = -5 \times 10^{-15}$ s) consistent with the formation of protein-surfactant and lipid-surfactant micelles. For $C_{12}E_{23}$ this did not occur and the S value suggests a further increase in f without proportionate increase in binding of surfactant.

DISCUSSION

The increases in R upon addition of surfactants could be due to: (i) increased particle hydration, (ii) the presence of adsorbed layers of surfactant molecules, (iii) LDL_2 aggregation, (iv) or increased particle assymetry. The lack of correlation between increases in R and polyethyleneoxide chain length, the increases caused by SDS and $C_{12}E_{10}$ with limited molecular dimensions, and the fact that maximum R values were reached before monolayer adsorption was complete (Tucker et al 1982) mean that (i) and (ii) were not the sole causes of the increases. Aggregation can be rejected since up to 2000:1 the increases occurred without an increase in the polydispersity of the system; but increased particle assymetry is supported by both the PCS and sedimentation velocity data.

The increased assymetry could be due to a partial unfolding of the apolipoprotein B on the surface of the LDL_2 or due to intercalation of the surfactant with the surface layer leading to a shape change to accommodate the increased surface to volume ratio. We found that R increased by 50% in 4 M guanidine hydrochloride in agreement with Ikai (1975). Ikai also showed that subtilisin BPN' digested LDL_2 was not affected by guanidine, thus unfolding of apolipoprotein B can produce the changes observed. However, $C_{12}E_{23}$ and $C_{12}E_{10}$ increased the radius of digested LDL_2 (Fig. 4), but to a lesser extent than that for native LDL_2, supporting the view that intercalation of the surfactant is in part responsible for the increases. Sklar et al (1980) using a fluorescent technique have shown that amphiphiles are located in the surface layer of LDL_2. Further, the solubilization of membranes is preceded by saturation of the bilayers with surfactant (Helenius & Simons 1975). So both intercalation and partial unfolding of apolipoprotein B appear to be responsible for the increases in R.

The fall in R with some surfactants (Fig. 2) is deduced to be due to breakdown of LDL_2 into lipid-surfactant micelles and protein-lipid-surfactant micelles. This is based on the following: $C_{12}E_{10}$ at 8000:1 showed a fall in R by PCS (Fig. 2c) and the velocity sedimentation profile indicated a floating component was present at this ratio, whereas $C_{12}E_{23}$ did not decrease R and a floating component was

absent; Ikai (1976) has shown that 10–15% of lipid is lost from LDL_2 at 2000:1, SDS:LDL_2 and 100% at 4500:1; high concentrations of SDS, CTAB, $C_8PE_{9\cdot5}$ (Helenius & Simons 1975) and $C_{12}E_8$ (Watt & Reynolds 1980) delipidate LDL_2 forming lipid-surfactant micelles (typically 10 nm radius) and surfactant-apolipoprotein B micelles. The $C_{12}E_8$ apoprotein B micelle has a Stokes' radius of 15 nm (Zampighi et al 1980) in approximate agreement with the value of 13 nm for $C_{12}E_{10}$ at 8000:1 (Table 1). However, the SDS-apolipoprotein B is reported to have a radius of 24 nm (Steele & Reynolds 1979) by ultracentrifugation which is at variance with the value of 15·3 nm for SDS:LDL_2 at 8000:1 (Fig. 2d). The reason for this is not known although Steele & Reynolds did report instability problems with the apolipoprotein B.

The data suggest that surfactant HLB is important in determining their ability to rapidly delipidate LDL_2. Non-ionic surfactants with HLB values <14·6 and the ionic surfactants caused a fall in R which we interpret as due to delipidation of LDL_2. Umbreit & Strominger (1973) and Collins & Salton (1979) found that surfactants with HLB values of 12·5–14·5 are most effective in solubilizing membranes. Thus LDL_2 is responding like a biological membrane and this is supported by the fact that the fall in R starts at approximately one mole of surfactant per mole of phospholipid, a typical value for membranes (Helenius & Simons 1975). However our kinetic data suggest that surfactants with higher HLB values (e.g. $C_{12}E_{23}$, HLB 16·9) might delipidate membranes over prolonged periods (20 h). The reason for these differences is not known but it is interesting to speculate. The lower HLB surfactants tested are also those with the shortest hydrophilic chains, and so the smallest molecular areas (Walters et al 1981). They should therefore have the highest concentrations at the LDL_2 surface at saturation causing disruption and solubilization. The slow action of $C_{12}E_{23}$ could be due to slow replacement of $C_{12}E_{23}$ molecules by lower HLB impurities in the surfactant samples as occurs on polystyrene microspheres (Kronberg et al 1981). Alternatively, lipid molecules may slowly partition between surfactant-saturated LDL_2 and surfactant micelles (Stubbs & Litman 1978). This latter alternative also provides a possible explanation of the biphasic response of LDL_2 to $C_{12}E_{10}$ (Fig. 1b). The breakup of LDL_2 into surfactant-lipid micelles and surfactant-lipid-protein micelles causes an immediate decrease in R, and this is followed by a slower partitioning of lipids from the surfactant-lipid-protein micelles to the lipid-surfactant micelles.

Since LDL_2 and membranes respond to surfactants in similar ways, with appropriate experimental design LDL_2 might provide another model system to investigate relationships between surfactant structure and the rate and extent to which they affect biological membranes. Walters et al (1981, 1982), using rat intestine and goldfish, have shown that $C_{12-16}.E_{10-12}$ surfactants are most effective in increasing the rate at which some drugs penetrate biological membranes. Thus it will be interesting to see if surfactants at subdelipidating concentrations will facilitate the loading of LDL_2 with drug molecules.

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