Physicochemical Characterization of Protein-Free Low Density Lipoprotein Models and Influence of Drug Loading

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Received May 23, 1995; accepted September 29, 1995

Purpose. Drug free and drug loaded protein-free low density lipoprotein (LDL) models consisting mainly of phospholipids, cholesterol, cholesterol esters, and triglycerides in ratios found for physiological LDL have been prepared. Their physicochemical characteristics were compared with those of physiological LDL.

Methods. Different characterization methods were used: photon correlation spectroscopy, transmission electron microscopy, X-ray solution scattering, and ^1H nuclear magnetic resonance spectroscopy (NMR).

Results. Particle sizes are highly dependent on the preparation method and in particular on the homogenization conditions. Electron microscopy indicates that the size distributions of model systems are much broader than those of physiological LDL. The X-ray solution scattering patterns of the model systems display a temperature dependent maximum near 3.8 nm similar to that found in the patterns of physiological LDL. NMR indicates a comparable mobility of the lipid molecules in model particles and in physiological LDL. The influence of drug loading is similar to that found earlier for physiological LDL. In particular, the incorporation of the anticancer drug WB 4291 seems to have a fluidizing effect on the lipids in the core region of the particles.

Conclusions. The preparation method of LDL model systems is of crucial importance as only the solvent evaporation method yielded systems in the size range of physiological LDL with acceptable high lipid concentrations. The fluidizing influence of temperature and drug incorporation (WB 4291) may be a disadvantage in drug targeting.

KEY WORDS: LDL; LDL models; synchrotron radiation; X-ray solution scattering; NMR; solvent evaporation.

INTRODUCTION

Lipoproteins, especially low density lipoproteins (LDL), have been proposed as carriers in targeted delivery of lipophilic substances (e.g. of cytostatics to cancer cells) [1,2]. The rationale for the use of physiological lipid particles as drug carriers was to circumvent some of the difficulties encountered with other carrier systems described for the administration of poorly water-soluble drugs including drug leakage, burst effects, and fast recognition of carriers by the reticuloendothelial system (RES). The availability of LDL is, however, limited by their physiological origin and the ensuing infection risks (hepatitis, AIDS). Artificial lipid systems resembling LDL could, in principle, combine the advantages of physiological transport vehicles (e.g. longer circulation times due to non-recognition by the RES and receptor-mediated cellular uptake) with the availability of large supplies and considerably reduced infection risks.

Several such systems have already been described [3-7]. Microemulsions containing the four main components of LDL in comparably physiological proportions were prepared with mean particle sizes of 35-45 nm by injection at total lipid concentrations of about 0.15% (w/v) [3]. Sonicated microemulsions consisting of one type of cholesterol ester and one type of phospholipid (approximately 1% (w/v) total lipids) were prepared by Ginsburg et al [4] with particle sizes in the range of physiological LDL. Thermal transitions were observed to be dependent on the type of cholesterol ester and phospholipid used for preparation. LDL models as drug carriers were discussed by Seki et al. [5] and Peyrot et al. [6]. Seki et al. [5] describe artificial lipoprotein-like particles consisting of phospholipids and simple lipids prepared by probe sonication. In the supramolecular biovectors of Peyrot et al. [6] the lipoprotein lipid core was replaced by crosslinked natural polysaccharides to allow the incorporation of various drugs, especially amphiphilic and hydrophilic substances. Preparation of these particles requires several steps to obtain a polysaccharide core surrounded by an inner neutral or ionic lipid layer covered by an outer phospholipid layer. Protein-free analogues of LDL containing phospholipids and cholesterol oleate mostly in a ratio of 2:1 were described by Owens et al. [7]. Systems were prepared by microfluidization and extrusion with final concentrations of 1.5% w/v and 7-8% w/v, respectively. The extruded particles were smaller, addition of poloxamer 188 reduced the particle size of microfluidized systems in the range of physiological LDL. Biodistribution of microemulsions resembling the lipid phase of LDL prepared as described in [4] were studied in patients with acute leukemia. An increased removal from plasma was found compared to healthy subjects [8].

The LDL models in the present study were made to resemble physiological LDL as closely as possible and therefore consist mainly of phospholipids, cholesterol esters, cholesterol, and triglycerides in ratios found for physiological LDL. The aim was to obtain particle size distributions centered around 25 nm and lipid concentrations higher than in other systems [4,7]. Differences between these models and existing systems should also be based on the preparation method. Extensive ultrasonication (e.g. 5 h as described by Ginsburg et al. [4]) at increased temperature, possibly leading to degradation products, should be circumvented. The batch size should be significantly larger than for systems prepared by ultrasonication or injection.

MATERIALS AND METHODS

Preparation of LDL Models

Chemicals

Lipoid E80 (~80-85% phosphatidylcholine, ~10% phos-

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phatidylethanolamine, ~2% sphingomyeline; Lipoid KG, D-Ludwigshafen), cholesterol (USP quality 95%, Sigma, D-Deisenhofen), cholesterol oleate (60-80%, Aldrich, D-Steinheim), cholesterol palmitate (97%, Aldrich, D-Steinheim), triolein (65%, Aldrich, D-Steinheim), tripalmitate (95%, Fluka, CH-Buchs), soy bean oil (USP quality, Wasserfuhr, D-Bonn), sodium glycocholate (approx. 99%, Sigma, D-Deisenhofen), sodium oleate (purum pulv., Caesar + Loretz, D-Hilden), thiomersal (DAC quality, Caesar + Loretz, D-Hilden), dichloromethane (redestilled).

Methods

1. Melt-Homogenization

Molten lipid components were mixed by probe-sonication (Soniprep 150, MSE). Warm water (~80°C) was added to the clear lipid mixture and the lipids were dispersed in the water phase by ultrasonication. This premix was used for high pressure homogenization with a Micron Lab 40 (APV Gaulin). To prevent crystallization of the lipid components the homogenization equipment was heated (~80°C). Homogenization was performed at 800, 1200, and 1500 bar with and without addition of bile salt as co-emulsifier. After the last homogenization step the systems were filtered (0.45 μm).

2. Solvent Evaporation

The solvent evaporation method of Sjöström and Bergenståhl [9] was used. Lipid components were dissolved in dichloromethane. Bile salt or sodium oleate were added to the water phase as co-emulsifiers. The two phases were mixed in a ratio of about 1:9 (organic/aqueous) and predispersed by ultraturrax vortexing for about two minutes. This dispersion was homogenized and the organic solvent was evaporated under vacuum. The systems were filtered (0.2 μm).

Homogenization was performed either with a continuously working Microfluidizer (M-120E, Microfluidics) cooled with ice with a homogenization pressure of 1100-600 bar at the beginning, decreasing to 600-400 bar during homogenization, or a discontinuously working Micron Lab 40 (APV Gaulin) at 460, 800, and 1200 bar.

Preparation of physiological LDL

Physiological LDL (1.03-1.05 g/ml) were isolated from human plasma by ultracentrifugation at different solvent densities [15].

Drug Loading

Two anticancer drugs, the adriamycin derivative AD 32 and the N-mustard derivative WB 4291 (Boehringer Ingelheim, UK) were incorporated into physiological LDL as well as into model systems. AD 32 was a gift of Dr. C. Peterson, Karolinska Hospital, Stockholm, Sweden. Drug loading was performed according to the method of Masquelier et al. [10]. The reconstituted systems were prepared following the same method, except that solvent without drug was added to the lyophilized product. Drug concentration was determined by HPLC using a μBondapak-phenyl column (Waters Associates) with 60% acetonitrile, 40% ammonium formate pH 4.0 (0.2%) as mobile phase and a flow rate of 1.5 ml/min. AD 32 was assayed at a wavelength of 475 nm with a filter fluorescence detector (Gilson Model FL-1B fluorometer) equipped with an excitation filter No. 042523 (Gilson Medical Electronics, cutoff at 520 nm) and an emission filter No. 042535 with a transition maximum at 600 nm. WB 4291 was assayed by its absorbance at 297 nm (Shimadzu SPD-6A UV-detector).

Photon Correlation Spectroscopy

Particle size distributions were obtained at 90° and 20°C using a Malvern Zetasizer 3 (Malvern Instruments) at a wavelength of $\lambda = 630$ nm ($\lambda = 400$ nm in water) using the exponential sampling method [11]. Mean particle sizes by number ($n = 5$) are given as diameters in the text and were calculated from relative frequency distributions with linear size classes derived from relative frequency distributions computed with logarithmic size classes by the instrument.

Synchrotron Radiation X-ray Solution Scattering

X-ray scattering patterns were collected at 5°C, 20°C, and 38°C on the double focussing monochromator mirror camera X33 of the EMBL in HASYLAB [12] on the storage ring DORIS III of the Deutsches Elektronen Synchrotron (DESY) at Hamburg using a multiwire proportional chamber with delay line readout [13]. The range of scattering vectors $0.06 < s < 0.68\ nm^{-1}$ ($s = 2\sin\Theta/\lambda$; 2Θ:scattering angle, $\lambda = 0.15$ nm) was covered. Scattering patterns of physiological LDL were measured in two over-lapping parts to cover the range of scattering vector (s) from 10^{-3} to $0.7\ nm^{-1}$. The data were processed following the standard procedures [14].

Nuclear Magnetic Resonance Spectroscopy

NMR measurements were performed on an ARX-400 spectrometer (Bruker) at room temperature (RT) and 38°C. Systems were changed to a D_2O medium (D_2O containing 5% of the original amount of co-emulsifier for LDL models and D_2O containing 1.8 g/l NaCl and 0.1 g/l EDTA for physiological LDL) by passage through Sephadex G-25 M PD-10 columns (Pharmacia).

Experimental conditions for ^1H NMR experiments were: Larmor frequency: 400.132 MHz, 400 scans, 0 Hz exponential line broadening, 3.6 kHz spectral width, 2 sec pulse repetition. 3-(trimethylsilyl-)-propanesulfonic acid, sodium salt, (0 ppm) was used as internal standard ($T_1 = 3.2$ sec, determined by the inversion recovery method). For (semi-) quantitative analysis experiment conditions were changed to 12 sec pulse repetition and 200 scans.

Microscopy

Negative staining electron microscopy was performed on a Zeiss EM 10 at 60 kV. The systems were diluted 1:1000 with phosphate buffered saline before adsorption on carbon coated grids and stained with 1% uranyl acetate.

Transmission electron microscopy of freeze fractured replica was performed on a Phillips EM 300 at 80 kV. Freeze fracturing was performed with a freeze-fracture unit BAF

400 (Balzers). Fast freezing was done using a JFD 030 (Baltec Walluf) jet-freeze device.

Fractured samples were shadowed under 45° with platinum/carbon (layer thickness 2 nm) and under 90° with pure carbon for replica production. Replica were picked up on uncoated microscope grids.

RESULTS AND DISCUSSION

The main difficulty in preparing LDL models is to obtain the desired particle size (25 nm) at an acceptable lipid concentration. The quality of the resulting particles is highly dependent on the preparation method (Table I). The smallest particles obtained by melt-homogenization at the highest pressure (1500 bar) with bile salt as co-emulsifier had a much too large mean particle size of 93 nm (not mentioned in Table I).

Only the solvent evaporation method yielded model particles with diameters around 25 nm. The preparation of stable lipid dispersions by this method requires the addition of water-soluble co-emulsifiers like bile salt or sodium oleate, which are not natural components of LDL, to avoid the formation of highly viscous, gel-like systems.

The mean particle size depends very much on the equipment used for homogenization. Particles with rather narrow size distributions and mean particle sizes of 22-30 nm were obtained with the continuously working Microfluidizer with a homogenization time of 5 minutes, whereas homogenization with the discontinuously working Micron Lab 40 resulted in systems with broader distributions and larger mean sizes (Table I). This may be due to uncontrolled solvent evaporation during homogenization.

Only model systems prepared by solvent evaporation using the Microfluidizer which had an adequate particle size distribution were investigated in more detail.

Different parameters were varied during the preparation of these models as indicated in Table I. Doubling the amount of bile salt from 0.2 to 0.4% in the aqueous phase results in a reduction of particle size from 32 nm to 22 nm. Doubling the total concentration as well as using sodium oleate as co-emulsifier in the same molar concentration as bile salt resulted in systems with mean particle sizes in the range of physiological LDL.

The mean particle size of System V determined by PCS was 23 ± 0.3 nm after preparation and 20 ± 0.5 nm after 10 months storage at 4-8°C indicating that there is no pronounced change upon storage. Higher amounts of co-emulsifier increase the storage stability as illustrated in Fig. 1. The particle sizes of systems containing less than 0.4% bile salt (System II) increase from 27 nm to 44 nm after 2 months and to 100 nm after 12 months storage. The mean particle size of systems prepared with 0.4% bile salt or 0.25% sodium oleate as co-emulsifiers, or with twice the total concentration of the stable preparations with the size range of physiological LDL, stored at 4-8°C is stable for at least 10 months.

The mean particle size of System V loaded with 1.9 w% of AD 32 (22 ± 1.3 nm) or 9.3 w% of WB 4291 (23 ± 0.8 nm) measured directly after preparation is not significantly different from the unloaded system (20 ± 1.5 nm; 21 ± 0.6 nm). The increase in particle size, which the addition of these amounts of drug to the lipid particles could cause, is too small to be detected. Clear differences were, however, detected upon storage. The preparation containing WB 4291 were unstable with a drastic increase in particle size from 23

Table I. Chemical Composition of LDL Model Systems Prepared by the Solvent-Evaporation-Method (% by Weight)

System		PL %	Ch. %	Ch. oleate %	Ch. palmitate %	Triglycerides %	Additives %	Size (s.d. [nm])	z-Average (s.d. [nm])	Polydispersity (s.d.)
Human plasma LDL[a]		0.8		1.5–2.1		0.2	0.8 Protein	20–25		
Microfluidizer:										
System I		1	0.25	1	0.15	0.2 TO	0.2 BS	32 (2.1)	51 (0.4)	0.26 (0.007)
System II		1	0.25	1	0.15	0.2 TO	0.3 BS	27 (1.0)	42 (0.4)	0.28 (0.003)
System III		1	0.25	1	0.15	0.2 TO	0.4 BS	22 (0.7)	38 (0.2)	0.29 (0.006)
System IV		2	0.5	2	0.3	0.4 TO	0.8 BS	26 (0.7)	39 (0.8)	0.26 (0.011)
System V		2	0.5	2	0.3	0.4 SO	0.8 BS	23 (0.3)	34 (0.2)	0.24 (0.009)
System VI		1	0.25	1	0.15	0.2 TO	0.25 NaOl	27 (0.3)	48 (1.6)	0.33 (0.014)
System VII		1	0.25	1.61	0.24	0.2 TO	0.4 BS	28 (0.5)	41 (0.6)	0.25 (0.010)
System VIII		2	0.5	3.22	0.48	0.4 TO	0.8 BS	28 (0.4)	40 (0.5)	0.24 (0.014)
Micron Lab 40:	Pressure (bar)									
System IX	800	1	0.25	1	0.15	0.2 TO	0.4 BS	45 (2.6)	71 (1.4)	0.27 (0.010)
System X	1200	1	0.25	1	0.15	0.2 TO	0.4 BS	58 (1.9)	81 (0.5)	0.24 (0.007)
System XI	1200	1	0.25	1	0.15	0.2 TO	0.4 BS	91 (1.4)	129 (0.8)	0.23 (0.007)
System XII	800	1	0.25	1	0.15	0.2 TO	0.25 NaOl	49 (1.5)	82 (0.8)	0.28 (0.004)
System XIII	460	1	0.25	1	0.15	0.2 TO	0.35 NaOl	44 (4.8)	64 (0.3)	0.28 (0.009)
System XIV	460	1	0.25	1	0.15	0.2 TO	0.35 NaOl	59 (5.7)	109 (2.6)	0.33 (0.003)

Abbreviations: PL = Phospholipids (Lipoid E 80 for all models); Ch. = Cholesterol; SO = Soybean Oil; TO = Trioleine; BS = Bile Salt; NaOl = Sodium Oleate.

[a] Data of physiological LDL investigated in [15].

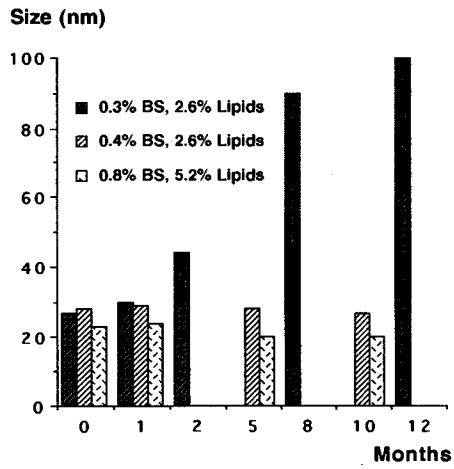

Fig. 1. Change in the approximated mean particle size as a function of storage time for different co-emulsifier (bile salt) and lipid concentrations.

nm to >700 nm after one month. The large particles could be easily visualized and the system broke down completely indicating that the increase in particle size is not only due to a recrystallization of the drug. After about 4 months of storage the mean particle size of all preparations involved in the drug loading process increased by a factor between 1.5 and 6. This instability was also observed with drug loaded preparations of physiological LDL.

Size distributions of model systems obtained by PCS and negative staining electron microscopy are broader than those of physiological LDL. The narrower size distributions of physiological LDL result partly from the purification process since only a rather narrow density fraction is separated from human plasma. The presence of co-emulsifiers and the absence of apolipoprotein in LDL model systems alter the surface properties of the particles and hence their behaviour in negative staining electron microscopy. Artificial particles are only poorly adsorbed compared to physiological LDL so that in the models mainly the larger particles (of about 70-110 nm) are visualized.

The existence of larger particles (up to 120 nm and more) in model systems is also demonstrated by size distributions obtained by PCS, especially the distribution by mass (Fig. 2). Transmission electron microscopy of freeze-fractured specimen of System V revealed the coexistence of small (about 20 nm) and large (in the range of 80 nm) particles in the sample.

Synchrotron Radiation X-ray Solution Scattering

X-ray scattering patterns of the present LDL models at RT are very similar to those of Ginsburg et al. [4]. At lower temperatures (5°C, 20°C) the X-ray scattering curves have a subsidary maximum near 3.8 nm (Fig. 3). This maximum disappears at higher temperature (38°C) and reappears upon cooling down to 20°C indicating a reversible structural change in the lipid particles. A similar temperature dependent maximum is found in the scattering curves of physiological LDL. Following the initial observations of Deckelbaum et al. [17,18] there is general agreement that this band reflects the segregation of a cholesterol-rich and a hydrocarbon-rich moiety of the cholesterol esters in the core of the particles at lower temperatures. The exact organization of this core in physiological LDL particles is, however, still controversial. Laggner et al. [19] postulate a model describing a thermal transition from a smectic-like structure with concentric spherical shells to a more disordered state. This conflicts with the model of Luzzati et al. [20] where a core with cubic symmetry undergoes a tetrahedral distortion correlated with the arrangement of the proteins on the surface of the LDL particles. The absence of maxima at lower angles ($s < 0.2\ nm^{-1}$) in the scattering curves of the model systems results from the broader size and shape distribution compared to physiological LDL as confirmed by electron microscopy.

Similar structural characteristics and temperature dependent structural changes in the original model system, reconstituted and AD 32 loaded particles (Fig. 4) indicate that loading with AD 32 does not seem to influence the order of the core lipids. Loading with WB 4291 has a clear effect even at low temperatures (Fig. 4). The disappearance of the thermal transition of the core lipids of the models after loading with WB 4291 indicates that the drug is taken up by the cholesterol(ester)-rich core and that its incorporation results in a significant lowering of the order/disorder thermal transition of the core lipids - mainly cholesterol esters. The data of the protein-free but drug loaded models clarify the interpretation of earlier observations on drug loaded physiologi-

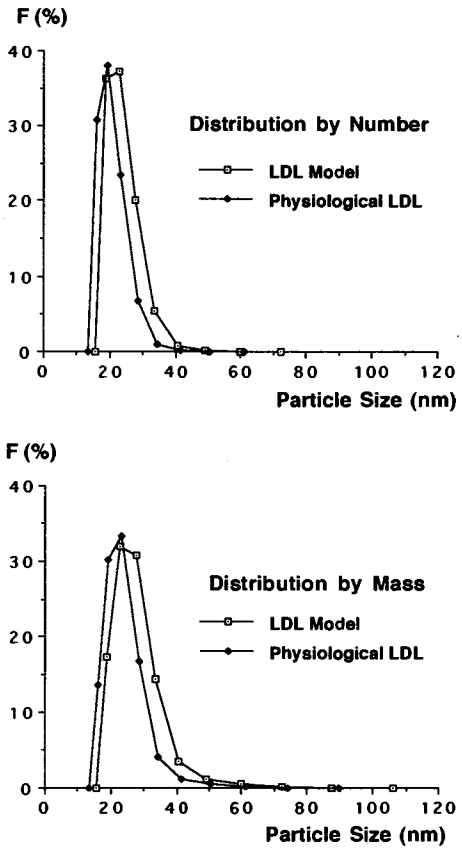

Fig. 2. Particle size distributions (relative frequency F (%)) of physiological LDL and model System V obtained by photon correlation spectroscopy.

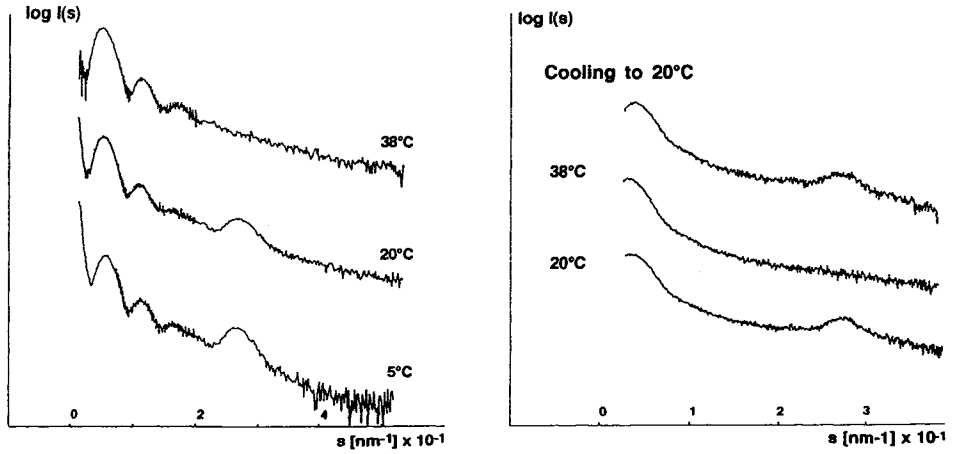

Fig. 3. Logarithm of the X-ray scattering intensity vs. scattering vector (s) for physiological LDL (left panel) and model System V (right panel) at different temperatures. The curves have been displaced along the ordinate for better visualization.

cal LDL [15] which were complicated by the complex structure of these particles and the uncertainties regarding the correct interpretation of their scattering pattern.

The very similar thermal and drug loading behaviour of physiological LDL and the model systems suggests that the latter may provide a way of obtaining some insight into the structure of LDL by specific deuteration of model components for neutron scattering, for instance.

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR spectra of drug free models (Fig. 5) are comparable to those obtained from physiological LDL particles [15]. The ratio of the integrated signal of the choline methyl-groups of the phospholipids (about 3.3 ppm) and of the signals related to methyl-groups of cholesterol esters, cholesterol and fatty acyl methylene-groups (0.5-1.5 ppm) is similar for models and physiological LDL indicating a similar mobility of the lipid components in the core region. As the signals of the cholesterol esters in the spectrum of the drug free System V are quite sharp, with linewidths of about 24 Hz (0.92 ppm) and 40 HZ (1.32 ppm), the molecules seem to have a certain motional freedom. ^{1}H NMR spectra of System V at RT and 38°C were used for (semi-) quantitative analysis of phospholipids (3.3 ppm) and lipid components (0.5-1.5 ppm). About 70% of the phospholipids at both temperatures and about 80% and 100% of the lipid components at RT and 38°C, respectively, were determined compared to data from chemical analysis. This indicates, that most of the model system components are detectable by NMR even at RT with parts of the lipids (mainly cholesterol esters) detectable only at 38°C due to the fluidizing effect of increased temperature.

The assignment of the resonances to the components of the preparation is in agreement with data from single components [15,16].

^{1}H NMR spectra of System V after drug loading (Fig. 5) were determined at RT and 38°C. The mobility of the core lipids of the original drug free system, reconstituted and AD 32 loaded models are comparable indicating no significant alterations due to the loading process itself or the addition of AD 32 molecules. Loading with WB 4291 clearly causes alterations. Several additional sharp signals attributable to the drug, e.g. signals corresponding to the aromatic part of the WB 4291 molecule (at about 7 to 8 ppm) and some in the region where signals from the lipid components also occur, are detected (Table II, Fig. 5d). This indicates that at least part of the WB 4291 molecules seem to have a high mobility. The AD 32 molecule also contains an aromatic region giving signals in the 7-8 ppm range determined in organic solvent ($CDCl_3$:CD_3OD 1:2, Table II). The other main chemical shifts overlap with strong signals of the lipids. The absence of signals in the 7-8 ppm range in the spectrum of the AD 32 loaded model (Fig. 5c) suggests that the motional freedom of AD 32 molecules is more restricted than that of WB 4291. Narrower linewidths at RT of the core lipid signals (~0.5-1.5 ppm) in the spectrum of the WB 4291 loaded model (18 Hz and 27 Hz for the two main signals in the 0.5-1.5 ppm range of the WB 4291 loaded sample of System V compared to 24 Hz and 40 Hz for the three other samples) also indicate a higher mobility of the core components in the WB loaded particles. At 38°C the linewidths of the core lipid signals of all spectra are similar as a result of increased motional free-

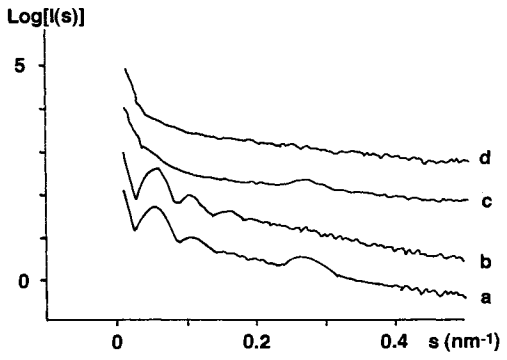

Fig. 4. Logarithm of the X-ray scattering intensity vs. scattering vector (s) for a) physiological LDL, b) WB 4291 loaded physiological LDL, c) original model System V, and d) WB 4291 loaded LDL model System V at 20°C. Note that the maximum near 3.8 nm in the WB 4291 loaded preparations has already vanished at 20°C. The reconstituted and AD 32 loaded preparations of both systems yield scattering curves that are very similar to those of the physiological LDL and the original model System V, respectively. The curves have been displaced along the ordinate for better visualization.

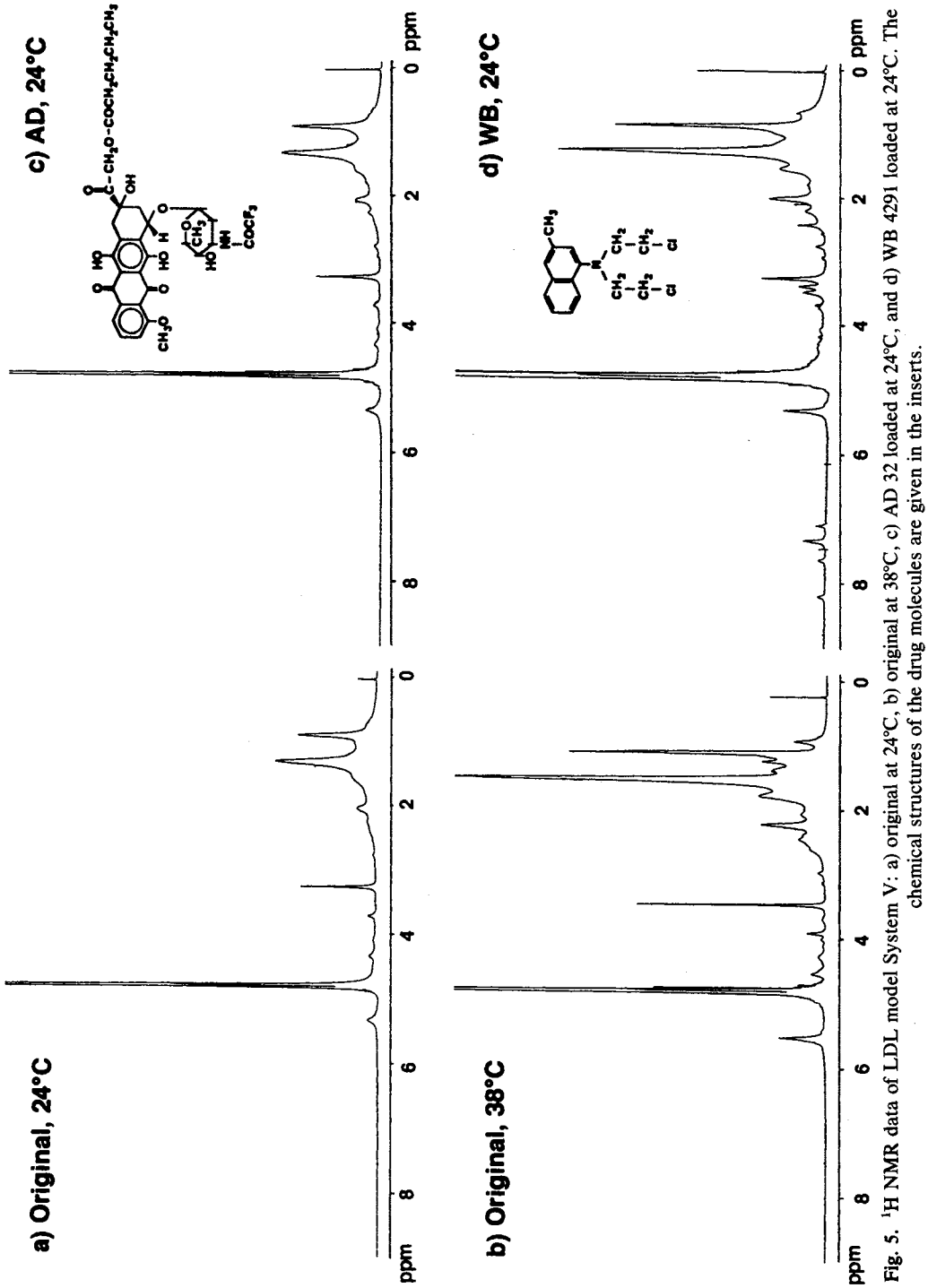

Fig. 5. ^{1}H NMR data of LDL model System V: a) original at 24°C, b) original at 38°C, c) AD 32 loaded at 24°C, and d) WB 4291 loaded at 24°C. The chemical structures of the drug molecules are given in the inserts.

Table II. ^{1}H NMR: Chemical Shifts (in ppm) of Drugs and Drug Loaded Model System V at 24°C

Drug free model	WB drug	WB loaded model	AD drug	AD loaded model
0.75	—	0.67	—	0.74
0.92	—	0.88	—	0.91
1.05	—	1.00	1.02[a]	1.00
1.32	—	1.25	1.35[a]	1.33
—	—	—	1.49[a]	—
1.58	—	1.55	—	1.60
—	—	—	1.72[a]	—
1.78	—	1.78	—	1.78
—	—	—	1.81[a]	—
2.04	—	2.01	—	2.06
2.26	—	2.19	2.17[a]	2.26
—	2.53	2.41	—	—
2.55	—	2.55	2.52[a]	2.53
—	—	—	2.64[a]	—
2.77	—	2.74	—	2.77
2.93	—	2.93	—	2.94
—	3.14[a]	—	—	—
3.28	—	3.26	3.32	3.28
—	3.35	3.37	—	—
—	3.63	3.47	—	—
3.72	—	3.70	—	3.71
4.07	—	4.04	4.09	4.08
4.34	—	4.32	—	4.32
broad water signal (~4.8)	4.93	broad water signal (~4.8)	4.93	broad water signal (~4.8)
5.34	—	5.31	—	5.33
—	—	—	5.44[a]	—
—	7.35	7.10	—	—
—	7.53	7.33	7.63	—
—	7.84	7.63	7.90	—
—	8.37	8.18	8.01	—

[a] Very small signal.

dom at higher temperatures. The ^{1}H NMR signals of the aromatic part of the AD 32 molecule could, however, not be observed even at higher temperature.

The difference between the two drug loaded samples suggests that about 90% of all WB 4291 molecules have a high mobility resulting in sharp, additional signals in the ^{1}H NMR spectra and in a narrowing of the lipid signals indicating the fluidizing influence of the WB 4291 loading on the core lipids. Since no specific signals due to the AD 32 molecules could be detected one must conclude that these molecules have a restricted motional freedom. In ^{1}H NMR spectra of small unilamellar vesicles (SUVs) existing in their lyotropic liquid crystalline state loaded with AD 32 or WB 4291 (unpublished results) only signals of the WB 4291 molecules could be detected. The WB 4291 molecules seem to have a high motional freedom also in SUVs indicating their predominant location (nearly 100%) in the disordered liquified hydrocarbon-chain region of the phospholipid bilayer. The AD 32 molecules seem to have a more restricted motional freedom, which might be due to intercalation of the drug molecules between the phospholipid molecules, i.e. perpendicular to the bilayer plane.

CONCLUSIONS

The difficulties generally encountered in preparing LDL models with the desired core lipid composition, particle size (~25 nm), lipid concentration above 5 w% and sufficiently large output volumes (~100 ml in this study) can be circumvented by homogenization using the solvent evaporation method. The absence of apolipoprotein and the addition of charged co-emulsifiers result, however, in particles with surface layers differing in their properties from those of physiological LDL as indicated by the altered adsorption behaviour of artificial particles on carbon coated grids. Drug loading of the model systems is possible in the same way as for physiological LDL and results in similar structural changes depending on the type of drug [15].

The coexistence of LDL model particles with other colloidal structures, such as bile salt containing vesicles, has to be investigated in more detail. Comparison of X-ray and NMR data of physiological and model LDL indicates, however, that the major portion of WB 4291 is bound to the model particles rather than to possibly coexisting colloidal emulsifier particles. The influence of the drug on the particle structure (fluidizing effect of WB 4291) promote fast drug release thereby limiting their use as drug targeting device. The high fluidity of the core lipids observed for all LDL and LDL model preparations at body temperature may in general question the usefulness of these carrier systems for a drug targeting, e.g. to cancer cells, if perfect sink conditions exist for an incorporated drug in the blood. To circumvent possible drug leakage problems after injection carrier systems containing lipid particles solid at body temperature but resembling LDL particles with respect to particle size have been developed [21] and are currently under investigation.

ACKNOWLEDGMENTS

The authors thank C. Peterson, M. Masquelier, S. Vitols and M. Pålsson, Department of Clinical Pharmacology, Karolinska Hospital, Stockholm (Sweden), for cooperation especially concerning isolation of physiological LDL, preparation of drug loaded samples, and determination of drug concentration, Dr. V. Wray and B. Jaschok-Kentner, National Research Center for Biotechnology, Braunschweig (Germany) for performance of NMR experiments, and Dr. D.-E. Lesemann, Federal Biological Research Center for Agriculture and Forestry, Braunschweig (Germany) for his assistance with negative staining electron microscopy. K.W. and A.G. also thank Pharmacia Oncology/Immunology, Helsingborg, for financial support.

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