FLUIDITY OF THE LIPID PHASE OF BOVINE SERUM HIGH DENSITY LIPOPROTEIN FROM FLUORESCENCE POLARIZATION MEASUREMENTS

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SUMMARY

The microviscosity of the lipid phase of bovine serum high density lipoprotein was determined by fluorescence polarization measurements on a lipophilic probe (1,6-diphenyl-1,3,5-hexatriene) dissolved in the lipoprotein. At 25°C the average microviscosity was 6.1 ± 0.5 poise, and the activation energy calculated from a plot of log η versus 1/T was 13±3Kcal/mole. A constant slope for the Arrhenius plot from 0 to 46°C indicated no apparent phase transitions in this temperature range.

Comparison of the present results with reported microviscosity values for rat lymphocyte membranes and liposomes [Shinitzky and Inbar (1974) J. Mol. Biol. 85, 603] indicates a more rigid environment of the probe in the high density lipoprotein system -- fluidity of the lipid appears to be considerably decreased in the lipoprotein relative to organic solvent or oil solutions of lipids, probably as a result of the anisotropic environment of the probe, high total cholesterol, and presence of protein in these particles.

Discovery in recent years of the fluid nature of cell membranes, and observation of the profound influence of the physical state of the lipids on various membrane functions [1], have led to the detailed investigation of the fluidity and organization of natural and artificial lipid containing systems. Serum lipoproteins represent relatively simple natural complexes that lend themselves very well to the investigation of protein-lipid interactions. The precise structure of these particles is not yet understood but it is in general accepted that at least the mammalian high density lipoproteins (HDL)[1] are spherical and appear to have a symmetrical distribution of components -- with lipid making up the core and with polar phospholipid head groups and most of the protein constituting the exterior shell of the particles [2]. The lipid portion of human HDL has been probed by proton [3,4] and ^{13}C [5] NMR spectroscopy, indicating in both cases a fluid nature for the lipids. The interpre-

[1] Abbreviations: HDL, high density lipoprotein; DPH, 1,6-diphenyl-1,3,5-hexatriene.

tation of the NMR results, however, leads to the assignment of markedly different degrees of fluidity for the lipids. Qualitative proton NMR results appear to indicate very high mobilities of the fatty acyl chains, comparable to motions of lipids dissolved in organic solvents [3,4]; whereas, the quantative treatment of ^{13}C-NMR data indicates considerably restricted motions of cholesteryl moieties and fatty acyl chains relative to the mobility of lipids dissolved in organic solvents.

The present communication reports the application of the fluorescence polarization technique of Shinitzky and coworkers [6-8] to the estimation of the microviscosity of the lipid component of bovine HDL, in an attempt to clarify the question of the degree of lipid fluidity in high density serum lipoproteins.

MATERIALS AND METHODS

Bovine HDL was prepared and its homogeneity was determined according to the procedures reported previously [9]. The fluorescent label, 1,6-diphenyl-1,3,5-hexatriene (DPH)[1] was obtained from Aldrich Chemical Co. Purity of DPH was examined by thin layer chromatography on Eastman Silica-Gel G plates using chloroform as solvent; a single fluorescent spot was obtained. DPH was incorporated into bovine HDL by exposing the lipoprotein solution in 0.1 M Tris-HCl, pH 8.0, 0.025% EDTA to a dispersion of DPH on Celite 545 (John Manville, Co.). Three ml of bovine HDL solution were equilibrated with 10 mg of DPH-Celite mixture, containing 1 mg DPH per 100 mg of Celite. After one hour the solid phase was removed by filtration through Millipore filters, and the solution was passed through a Sephadex G-25 column (1.5 x 20 cm). This procedure gave bovine HDL-DPH preparations containing less than one molecule of DPH per lipoprotein particle, and exhibiting the same structural properties as unlabeled bovine HDL (as judged from sedimentation volicity coefficients and CD spectra).

Fluorescence polarization measurements were performed with a dual channel SLM - fluorescence polarization instrument, using 366 nm exciting light, selected with a manochromator, and observing simultaneously fluorescent light

polarized parallel and perpendicular to the exciting plane polarized light, through Corning glass C3-74 filters with cut-off at 400 nm. Fluorescence polarization (p) and anisotropy (r) were calculated from the usual expressions using measured fluorescence intensities: $P = (I_{\parallel}/I_{\perp}-1)/(I_{\parallel}/I_{\perp}+1)$; $r = (I_{\parallel}/I_{\perp}-1)/(I_{\parallel}/I_{\perp}+2)$. Temperature was regulated with a thermostated circulating water bath manufactured by Forma Scientific. Lifetime measurements were performed on the cross-correlation phase fluorometer designed by Spencer and Weber [10]. Differences in lifetimes determined by the phase and modulation method were less than 10%, indicating low heterogeneity of the probe environments. Average lifetimes were simply taken as the arithmetic mean of the phase and modulation lifetimes.

Microviscosities (η) were determined by using Perrin's equation: $r_0/r = 1 + C(r)\frac{T\tau}{\eta}$; where r_0 is the limiting anisotropy for DPH, given as 0.362 by Shinitzky and Barenholz [7]; τ is the fluorescent lifetime; T and η, have the usual meaning; and C(r) is a parameter related to the rotational volume and shape of the fluorescent probe. Calibration curves for DPH using paraffin oils at different viscosities give essentially linear relationships of r_0/r versus $T\tau/\eta$ with C(r) values near $(8.6 \pm 0.4) \times 10^5$ poise $deg^{-1}s^{-1}$ as reported by Shinitzky and Inbar [8]. Rotational relaxation times, $\rho_h = 3V\eta/RT$ (V, represents the total rotational volume of the fluorescent particle; R is the gas constant) were obtained from plots of 1/p versus T/η employing the Perrin relationship as applied by Weber to macromolecules [11]. The activation energy was calculated from the expression $\eta = Ae^{\Delta E/RT}$, typical for pure liquids, using log η against 1/T plots [6,8].

RESULTS AND DISCUSSION

Apparent rotational relaxation times for bovine HDL were calculated from plots of 1/p versus T/η shown in Fig. 1. When T/η was changed by isothermic variation of the viscosity of the solution by adding solid sucrose [11], a linear plot with a slope near zero was generated. The rotational relaxation time calculated from these results and an average lifetime of 8.3 nsec, is

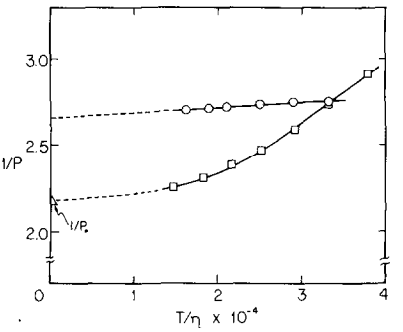

Fig. 1. Plots of inverse of fluorescence polarization against temperature over viscosity (°K/poise). T/η varied by isothermic (25°C) addition of sucrose (—O—O—O—); T/η varied by changing temperature from 0 to 35°C (—□—□—□—); (Δ) literature value for the limiting polarization of DPH.

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630 ± 60 nsec at 25°C, in agreement with previously reported results obtained with other fluorescent probes attached to bovine HDL [12]. Temperature variation gives a much lower apparent ρ_h, 35 nsec at 25°C, indicating the existence of temperature activated rotations of the probe. Since the depolarization effects due to Brownian rotations of the entire lipoprotein occur in a time scale almost two orders of magnitude longer than the fluorescent lifetime of the probe, as indicated by the sucrose addition experiment, any significant changes in fluorescence polarization observed with the DPH labeled lipoprotein can be attributed to motion of the probe itself and to influences of the immediate environment upon it (range of approximately 10 to 20Å [13]).

Figure 2 shows the temperature dependence of the microviscosity of DPH labeled bovine HDL, by means of a plot of log η versus 1/T. The linear relationship over the temperature range from 0 to 46°C indicates no observable phase transitions and yields an activation energy for the rotational motion of the probe of 13 ± 3Kcal/mole.

Fluorescence polarization and microviscosity results are summarized for a few temperatures in Table I, together with some representative data reported by Shinitzky and coworkers (6,8) for cell membranes and artificial lipid systems with and without added cholesterol. Bulk viscosities of some organic

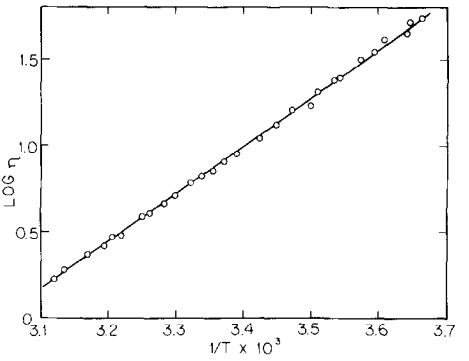

Fig. 2. Temperature dependence of microviscosity, given in terms of log η versus 1/T, for bovine HDL labeled with DPH.

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solvents and oils are included as a reference [14]. Comparing viscosity values at 25°C, it is clear that organized lipid phases such as cell membranes and lecithin liposomes have considerably lower fluidity in their liquid crystalline state than dilute solutions of lipids in organic solvents where the bulk viscosity would be similar to that of the pure solvent. Miscroviscosity in lecithin liposomes, rat lymphocytes, and egg lysolecithin micelles is comparable within a factor of two to the microviscosity in triolein (1.3 poise). Viscosities below the liquid crystal to gel transition temperature for dipalmitoyl lecithin in water (transition temperature 39°C), or viscosities determined after the addition of cholesterol to liquid crystalline lipid systems such as lecithin liposomes, are quite high, 9.4 and 12 poise at 25°C, respectively. Bovine HDL, with a microviscosity of 6.1 ± 0.5 poise has an intermediate fluidity indicative of a liquid lipid phase which is nevertheless considerably restricted in mobility. The location of the rod-shaped probe in the lipoprotein interior is not known, but it can be inferred from the viscosity results that DPH is probably not located in an isotropic triglyceride environment as fluid as some oils (e.g., linseed oil and triolein). Rather, the relatively low fluidity of bovine HDL lipids measured by fluorescence polarization appears to reflect an anisotropic environment of the probe (such as ordered

Table I

Comparison of fluorescence polarization and microviscosity data for bovine HDL with other lipid systems

System	Temperature (°C)	Polarization[a]	Microviscosity (poise)
Bovine HDL	10	0.420	19
Bovine HDL	25	0.346	6.1
Bovine HDL	40	0.296	2.6
Triolein[b]	25		1.3
Egg lysolecithin	25		0.77
Egg lecithin	25		1.2
Egg lecithin-cholesterol (1.5:1)	25		12
Dipalmitoyllecithin	25		9.4
Rat lymphocytes[c]	25	0.275	2.8
Diethyl ether[d]	25		0.0022
Chloroform	25		0.0054
Linseed oil	30		0.33
Castor oil	25		6.5

[a]Polarization data are only given where DPH was used as the probe.

[b]Microviscosity results from triolein through dipalmitoyllecithin were taken from Cogan et al. [6].

[c]Data for rat lymphocytes were taken from Shinitzky and Inbar [8]

[d]Bulk viscosity values for the organic solvents and oils were obtained from the Handbook of Chemistry and Physics [14].

and anchored acyl chains), the high total cholesterol content of the lipoprotein, and perhaps the influence of the protein component on the organization of the lipid phase.

Regarding the NMR reports on the fluidity of HDL lipids, our results do not agree with the interpretation of proton NMR high resolution spectra [3,4] that suggest very high fluidity for the lipid components, comparable to organic solutions of the lipids. The ^{13}C-NMR results [5] are in closer agreement with our microviscosity data. Within the frame of approximations used in reference [5] and in the fluorescence polarization technique [6-8], the fluidity values obtained by us (2.6 poise at 40°C) are in general agreement with the time scale of rotational correlation times estimated from ^{13}C T_1 of the cholesterol

moiety and also suggested for acyl chains [5].

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