MICROVISCOSITY OF THE LIPID DOMAINS OF NORMAL AND HYPERCHOLESTEROLEMIC VERY LOW DENSITY LIPOPROTEIN*

Francis J. Castellino,+ J. Kerry Thomas, and Victoria A. Ploplis
Department of Chemistry, The University of Notre Dame,
Notre Dame, Indiana 46556

Received March 4,1977

SUMMARY: Very low density lipoprotein (VLDL) has been isolated from normal (n) and dietary-induced hypercholesterolemic (hc) rabbits. Incorporation of the fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene into the lipid domains of both n VLDL and hc VLDL allowed assessment of the fluidity characteristics of these particles, utilizing fluorescence polarization techniques. Over the temperature range of 5° - 45°, the lipid region of n VLDL consists of an invariant phase, characterized by a microviscosity, η, at 30° of 0.6 ± 0.2 poise and a fusion activation energy, ΔE, of 7.6 ± 1.5 kcal/mole. The lipid region of hc VLDL, over the same temperature range, also is invariant and is characterized by a value of η at 30° of 4.6 ± 0.3 poise, and a ΔE of 7.8 ± 1.5 kcal/mole. Thus, large differences in the fluidit of the lipid in n VLDL and hc VLDL are evident, most probably due to the greatly increased content of cholesterol esters in hc VLDL, compared to n VLDL.

It is well known that hypercholesterolemia is induced in rabbits upon intake of cholesterol rich diets (1,2). As result, the nature of the serum lipoproteins, especially very low density lipoprotein (VLDL), is considerably altered (1). In hypercholesterolemic (hc) rabbits, greatly elevated concentrations of VLDL exist (3), which possess strikingly different lipid compositions, when compared to VLI isolated from normal rabbits (3,4). In particular, large increases i cholesterol ester content and large decreases in triglyceride content are observed in hc VLDL, when compared to normal (3,4).

Previous work (5) has shown that incorporation of cholesterol into phospholipid vesicles results in a marked increase in the

*These studies were supported by Grant HL-19982 from the National Institutes of Health.

+Recipient of a Research Career Development Award, HL-70717, from the N.I.H. To whom to address correspondence.

ISSN 0006-291X

rigidity and internal order of the resulting vesicle. As part of our recent studies on the role of circulating lipoproteins in blood coagulation processes (6), we have assessed the very low density lipoproteins from normal and hypercholesterolemic rabbits in regard to their comparative stimulation of prothrombin activation (7). In order to attempt to account for the differences observed, we wish to study structural differences in the lipid phases of these classes of VLDL. In the present communication, we report on the hydrocarbon fluidity, possible phase transitions, and fusion activation energies of the lipid phases in normal (n) and hyperchloesterolemic (hc) VLDL by fluorescence polarization techniques.

MATERIALS AND METHODS

Animal treatment. Commerically purchased New Zealand white rabbits were maintained on Purina® rabbit chow for 2-3 weeks. After this time, the rabbits were fasted for 3-4 hours to allow chylomicron clearance, and the blood removed by suction from an ear vein. The serum subsequently obtained was utilized for preparation of n VLDL.

In order to induce dietary hypercholesterolemia, another group of animals were fed rabbit chow, supplemented with 2% cholesterol (U.S. Biochemical Corporation, Cleveland, Ohio). When the total serum cholesterol level exceeded 1500 mg/100 ml, the animals were bled as above, and the serum obtained.

Isolation of lipoproteins. The serum obtained from the above rabbits was utilized to prepared n VLDL and hc VLDL (density <1.019 g/ml). The serum was diluted (1:2 for normal and 1:6 for h.c.) with 0.15 M NaCl, pH 7.4 (density = 1.005 g/ml) (8) and centrifuged in a Beckman Model L5-65 ultracentrifuge, utilizing a Beckman 60 Ti rotor, at 37,000 rpm and 4^{o} for 16 hours. The VLDL was then removed from the top 2 ml of the centrifuge tubes. After isolation, the VLDL samples were dialyzed against 0.005 M Tris-HCl, 0.15 M NaCl, pH 7.4,

at 4°. The resulting samples were concentrated to a suitable volume by pressure filtration and stored under N_2 at 4°.

Fluorescence polarization analysis. The VLDL preparations were adjusted to ca. 0.3 μM phospholipid (9) and labeled to the extent of ca. 0.05 μM with 1,6-diphenyl-1,3,5-hexatriene (DPH), essentially as described by others (5). After full incorporation of the dye (ca. 2-3 hours at 35°), the samples were diluted to 0.01 μM in phospholipid, prior to analysis.

Fluorescence polarization measurements were performed at various temperatures utilizing an Elscint Model MV-1a fluorescence polarization apparatus. DPH was excited at 365 nm and for emission, a cut-off filter for wavelengths below 418 nm was used. The methodology was similar to that described by Shinitzky and Barenholz (10). In all cases, the samples were continuously diluted until a constant polarization value was obtained in order to correct for possible depolarization due to light scatter. Microviscosity values were determined from the modified Perrin equation, using the relationship $r_o/r = 1 + [kT\tau]/[\eta V_{(r)}]$. Here, r_o is the limiting anisotropy; r, the measured anisotropy; k, Boltzmann's constant; T, the absolute temperature; τ, the lifetime of the excited state; η, the viscosity of the medium; and $V_{(r)}$, a parameter relating to the molecular shape of the fluorescent probe. Based on the above equation, a standard curve of the degree of depolarization (r_o/r) versus the quantity $\frac{T\tau}{\eta}$ was constructed in white oil U.S.P. 35 (10). Measurement of the value r_o/r (obtained as r/r_o directly from the instrument), allowed calculation of η, with knowledge of T and τ. Values of τ were obtained at various temperatures by means of a Cerenkov pulse produced by a Van der Graaf electron accelerator, utilizing the methodology previously described (11).

Analytical procedures. Total and free serum cholesterol levels

were determined as described by Crawford (12), except that we evaluated cholesterol content based on a standard cholesterol curve, rather than on a single level of cholesterol.

RESULTS AND DISCUSSION

In this study, fluidity properties of VLDL, isolated from normal rabbits (serum total cholesterol = 95 ± 5 mg/100 ml) and hypercholesterolemic rabbits (serum total cholesterol = 2200 ± 100 mg/100 ml), have been determined. In the isolated lipoproteins, n VLDL contained 5.6 ± 0.3% free cholesterol and 10.1 ± 0.9% esterified cholesterol; whereas, hc VLDL contained 19 ± 1% free cholesterol and 52 ± 2.5% esterified cholesterol. This confirms earlier work (3,4) showing the existence of increased levels of total cholesterol in VLDL from hypercholesterolemic rabbits, and attests to the appropriateness of our preparations. Fluorescence polarization measurements of DPH, dissolved in the lipid phase of n VLDL and hc VLDL, coupled with fluorescence lifetime (τ) determinations, allowed calculation of microviscosity values of 0.6 ± 0.2 poise to be determined for n VLDL at 30° and 4.6 ± 0.3 poise to be determined for hc VLDL, at the same temperature. This large increase in lipid microviscosity for hc VLDL, compared to n VLDL, is likely due to the greatly increased levels of cholesterol and cholesterol esters in hc VLDL. Previous studies have shown that addition of cholesterol to egg lecithin and dipalmitoyllecithin vesicles greatly increased the microviscosity of the lipid phase (5). For example, the microviscosity of dipalmitoyllecithin at 25° increased approximately 5.6 fold in dipalmitoyllecithin-cholesterol (3:1) mixtures (5). This microviscosity change, observed in synthetic bilayer vesicles, is in line with the change which we observe upon physiological incorporation of cholesterol and cholesterol esters in a circulating particle (VLDL). Thus, the fact that VLDL consists of a mixture of peptides, as well as differing

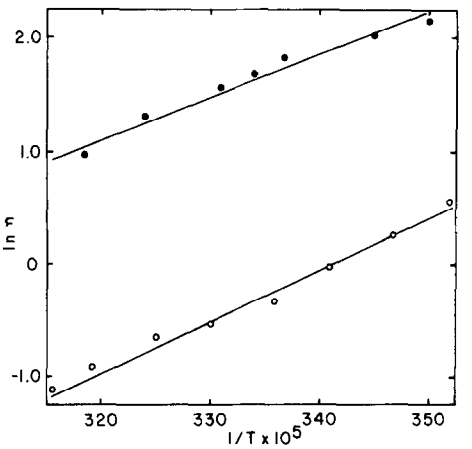

Figure 1. The temperature dependence, plotted as 1/T, of the lipid microviscosity, plotted as ln η, of normal VLDL (O), and hc VLDL (●). Fusion activation energies, ΔE, were calculated from the slopes of these particular curves.

classes of lipids does not alter the fact that cholesterol and cholesterol esters incorporation greatly increases the rigidity of the lipid phase. In addition, it had not previously been shown that cholesterol esters acted to increase the lipid microviscosity, as did unesterified cholesterol. Since most of the cholesterol which is incorporated into hc VLDL is in its esterified form, it is likely that cholesterol esters behave qualitatively similar to cholesterol with regard to this property.

The temperature dependence of the microviscosity of n VLDL and hc VLDL was plotted according to the equation (10):

$$\eta = Ae^{\Delta E/RT}$$

where η is the microviscosity; R, the gas constant; T, the absolute temperature; and ΔE, the fusion activation energy. These plots are shown in Figure 1. Each lipoprotein consists of a constant liquid crystalline phase over the temperature range of 5^{o} - 45^{o}. This phase is characterized by a ΔE of 7.6 ± 1.5 kcal/mole for n VLDL, and 7.8 ± 1.5 kcal/mole for hc VLDL. No phase transitions were observed in

either sample. Thus, this particular property is similar for both n and hc VLDL.

In conclusion, we have shown that the fluidity properties of the lipid phase of n VLDL and hc VLDL are considerably different. This difference may have relevance to the differing abilities of n and hc VLDL to substitute for phospholipid in blood coagulation activation mechanisms, as well as other functional properties of this important lipoprotein class.

REFERENCES

1. Schumaker, V. N. (1956) Am. J. Physiol. 184, 35-42.
2. Kritchevsky, D., Marcucci, A. M., Salatta, P., and Tepper, S. A. (1969) Med. Exp. 19, 185-193.
3. Camejo, G., Bosche, V., Arreaza, C., and Mendez, H. C. (1973) J. Lipid Res. 14, 61-68.
4. Rodriguez, J. L., Ghiselli, G. C., Torreggiani, D., and Sirtori, C. R. (1976) Atherosclerosis 23, 73-83.
5. Cogan, U., Shinitzky, M., Weber, G., and Nishida, T. (1973) Biochemistry 12, 521-528.
6. Bajaj, S. P., Harmony, J. A. K., Martinez-Carrion, M., and Castellino, F. J. (1976) J. Biol. Chem. 251, 5233-5236.
7. Ploplis, V. A., and Castellino, F. J. (1977) Fed. Proc., in press.
8. Havel, J. R., Eder, H. A., and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345-1353.
9. Lowry, O. H., and Lopez, J. A. (1946) J. Biol. Chem. 162, 421-428.
10. Shinitzky, M., Dianoux, M.-C., Gitler, C., and Weber, C. (1971) Biochemistry 10, 2106-2113.
11. Wong, M., Gratzel, M., and Thomas, J. K. (1976) J. Am. Chem. Soc. 98, 2391-2397.
12. Crawford, N. (1958) Clin. Chim. Acta 3, 357-367.