Lipid domain in cancer cell plasma membrane shown by ^1H NMR to be similar to a lipoprotein

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Human blood lipoproteins have been characterised by ^1H NMR methods and chemical analysis, and comparisons made with the properties of the triglyceride-rich plasma membrane domain found in cancer cells. By means of selective and non-selective T_1 experiments, the lipids in HDL and LDL are shown to be in diffusive exchange. In contrast, the lipids of chylomicra and VLDL do not exhibit lipid diffusion, and therefore resemble the neutral lipids of cancer cell plasma membranes. 2D scalar correlated NMR (COSY) spectra of cancer cells or solid tumours are similar to those obtained from VLDL and LDL. The long T_2 relaxation value observed for neutral lipid methylenes in metastatic cancer cells (> 300 ms) was not observed for any of the 4 lipoproteins studied. None of the lipoprotein classes gave a T_2 longer than 250 ms.

Lipoprotein *2D NMR* *Triglyceride* *(Cancer cell)* *Plasma membrane*

1. INTRODUCTION

High-resolution ^1H NMR spectra generated from lipids in the plasma membrane of cancer cells [1] are an indication of the biological status of those cells. The spectra have been used to monitor metastatic potential [2] and the onset of drug resistance (unpublished).

Two dimensional (2D) NMR [3] and chemical analysis of highly enriched plasma membranes have identified the membrane domain in question to be rich in triglyceride, with varying amounts of cholesterol ester [4]. Until recently triglyceride and cholesterol ester were considered unusual components of membranes [5].

The neutral lipid in the plasma membrane has an NMR linewidth of 10–30 Hz, and the application of selective and non-selective T_1 techniques [6] has detected no diffusive exchange with other lipids in the conventional bilayer [7]. Furthermore, the neutral lipids are able to tumble isotropically and independently of other membrane components [7].

Human lipoproteins provide a range of protein and lipid compositions and many of their properties are well documented [8–10]. These particles have been studied by T_1, T_2 and 2D NMR methods to determine if they provide a good model for the triglyceride rich plasma membrane domain in cancer cells.

2. MATERIALS AND METHODS

2.1. *Preparation of lipoprotein fractions*

Human blood (200 ml) was allowed to clot at room temperature for 2 h. Chylomicra were separated by flotation [11] and the remaining lipoprotein fractions were isolated according to Hatch and Lees [12]. All samples were dialysed against EDTA-Na_2 in D_2O (0.18%, pH 7.4) followed by 0.9% NaCl/0.01% NaN_3 in D_2O prior to NMR analysis.

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2.2. *NMR spectroscopy*

^{1}H NMR spectra were recorded at 37°C using a Bruker WM-400 spectrometer equipped with an Aspect 2000 computer. 2D scalar correlated spectroscopy (COSY) experiments [3] and T_1 and T_2 measurements [7] have been described previously.

2.3. *Cell culture*

The cell line used for NMR analysis was the rat mammary adenocarcinoma line, J clone. Cells were grown in RPMI-1640 medium as described [7].

2.4. *Chemical analyses*

The protein content of the lipoprotein suspensions was obtained with the BioRad protein assay kit using bovine serum albumin as standard. The method of lipid extraction from dialysed lipoprotein samples was that of Gottfried [13], carried out at room temperature and in the presence of BHT (2,6-dibutyl-*p*-cresol). Non-lipid contaminants were removed by the method of Williams and Merrilees [14]. The lipid content of the extract was obtained by weighing an aliquot of the total lipid extract. The free cholesterol content of the lipid extracts was determined using an enzymic fluorometric method [15]. Total cholesterol content was measured by the same fluorometric method after saponification of the samples. Cholesterol ester content was calculated as the difference between total and free cholesterol content. Lipid phosphorus was determined on the total lipid extracts by a colorimetric method [16]. Triglyceride content was determined by using a colorimetric kit (Sigma technical bulletin no.405 [1983]).

3. RESULTS

The 4 main classes of human lipoproteins were isolated. Chemical analyses of protein and lipid content of the lipoprotein subclasses indicate that the fractions isolated are comparable with the literature. Cholesterol ester levels are lower than expected, and protein levels exceed those previously reported [8–10] (table 1). No attempt was made to purify the fractions. The sizes of the particles in each lipoprotein fraction, as determined by electron microscopy, are within the published values (table 1).

The 400 MHz ^{1}H NMR spectra of these 4 fractions are shown in fig.1 along with that obtained for a suspension of J clone cells. All spectra exhibit characteristics of a lipid spectrum with the -C = C-, $-N(CH_3)_3^+$, $(-CH_2)_n$ and CH_3 groups at 5.2, 3.2, 1.2 and 0.85 ppm, respectively [7]. However the HDL spectrum is very broad and it appears that the lipid spectrum is superimposed on a broad protein spectrum [17].

T_1 experiments were undertaken to determine if diffusion was taking place between the lipids. Based on the experiments by Brown and Davis [6],

Table 1

Chemical analysis of lipoprotein fractions

	% by wt[a]			
	Chylomicra	VLDL	LDL	HDL
Free cholesterol	0.15 ± 0.04	1.2 ± 0.12	2.2 ± 0.07	0.01 ± 0.0
Cholesterol ester	ND	3.1 ± 0.06	6.9 ± 0.30	0.1 ± 0.02
Phospholipid	5.2 ± 1.8	28.8 ± 0.33	41.7 ± 0.22	1.7 ± 0.23
Triglyceride	61.8 ± 3.9	49.3 ± 2.7	15.6 ± 0.59	0.3 ± 0.02
Protein	32.9 ± 6.7	17.6 ± 1.8	33.6 ± 3.7	97.9 ± 0.70
Particle size[b] (nm)	26–274	22–54	8–22	3–4

[a] The values represent the mean ± SE of 2 experiments except for chylomicra which is the mean ± SD for 3 experiments

[b] Particles were measured by negative staining electron microscopy. The preparations were stained with 1% ammonium molybdate solution (pH 8) and the electron micrographs obtained with a Philips 400 EM, operating at 100 kV and a maximum working magnification of 92000

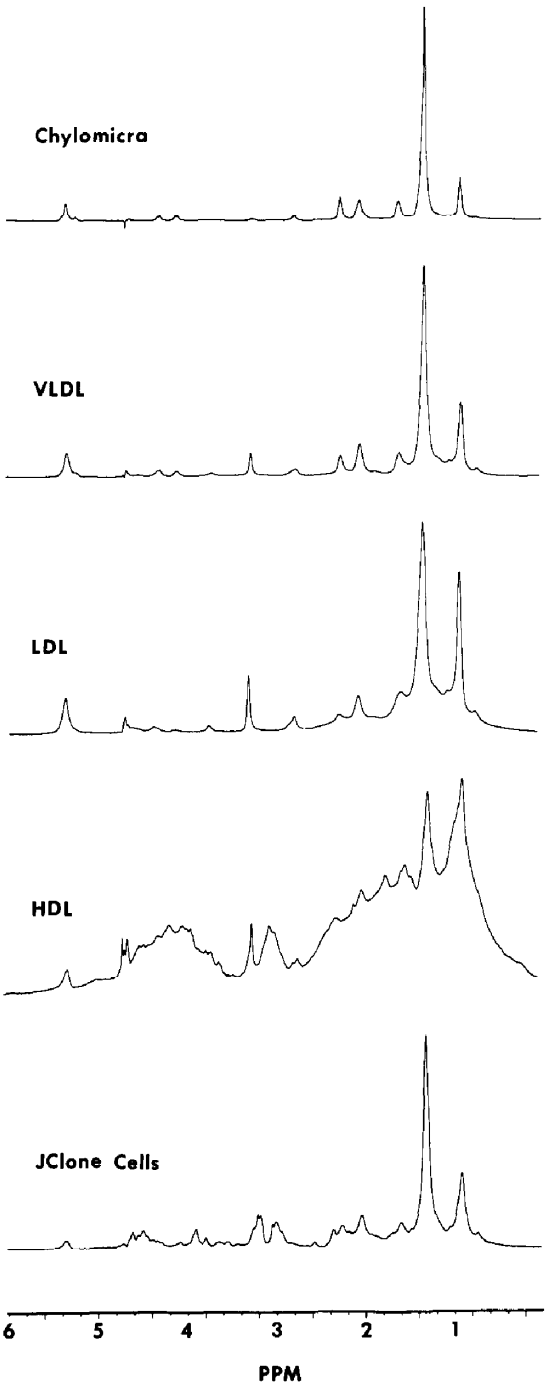

Fig.1. 400 MHz ^{1}H NMR spectra of chylomicra, VLDL, LDL, HDL and of the rat mammary adenocarcinoma cell line, J clone. Data were recorded at 37°C, with the sample spinning, using a sweep width of 4000 Hz. A line broadening of 3 Hz was applied.

the observation of single-exponential behaviour along with identical T_1 values for selective and non-selective inversion recovery experiments indicates that no exchange exists between protons contributing to the broad methylene resonance at 1.2 ppm [7]. This was the case for both chylomicra and VLDL, which had T_1 values of 0.55 ± 0.02 and 0.50 ± 0.02 s, respectively. In contrast LDL and HDL methylenes did not exhibit single exponential behaviour. Instead an initial rapid decay was observed due to transfer of magnetisation between the component lipids whilst the slope at longer times averages out over all orientations. Non-selective T_1 values for LDL and HDL were 0.37 ± 0.02 and 0.34 ± 0.02 s, respectively. The result for HDL and LDL was typical of that reported by Brown and Davis [6] for phospholipids.

Application of the CPMG pulse sequence to measure T_2 relaxation [7] showed that none of the 4 classes of lipoproteins has a T_2 in excess of 250 ms.

2D NMR clearly identifies the main component of chylomicra and VLDL to be triglyceride (fig.2). Triglyceride exhibits a unique cross-peak (G′) linking resonances at 4.1 and 4.3 ppm resulting from the geminal coupling of protons on carbons 1 and 3 of the glycerol backbone (structure, fig.2) [4]. This resonance is 0.1 ppm downfield from the corresponding glycerol resonance in the phosphatidylcholine spectrum [3]. All the cross-peaks marked in the chylomicra and VLDL spectra (fig.2) may be accounted for by triglyceride except for that denoted Z, which is from coupling between the methyl and methine protons of the alkyl side chain of the cholesterol molecule.

The spectra of LDL and HDL (fig.3) differ from the chylomicra and VLDL in that the unique triglyceride cross-peak G′, between 4.1 and 4.3 ppm is absent. However, a similar pattern is evident at a lower chemical shift and this may be due to phospholipid head groups or to protein. The acyl chain resonances A and B are less intense, and cross-peaks E and F from methylenes close to the carbonyl are absent altogether. The spectra of LDL and HDL are generally broader (as is also evident in the 1D spectra, fig.1) which is a reflection of their increased protein content (table 1).

The 2D COSY spectra of the lipoproteins (figs 2,3) may be compared with that obtained from a

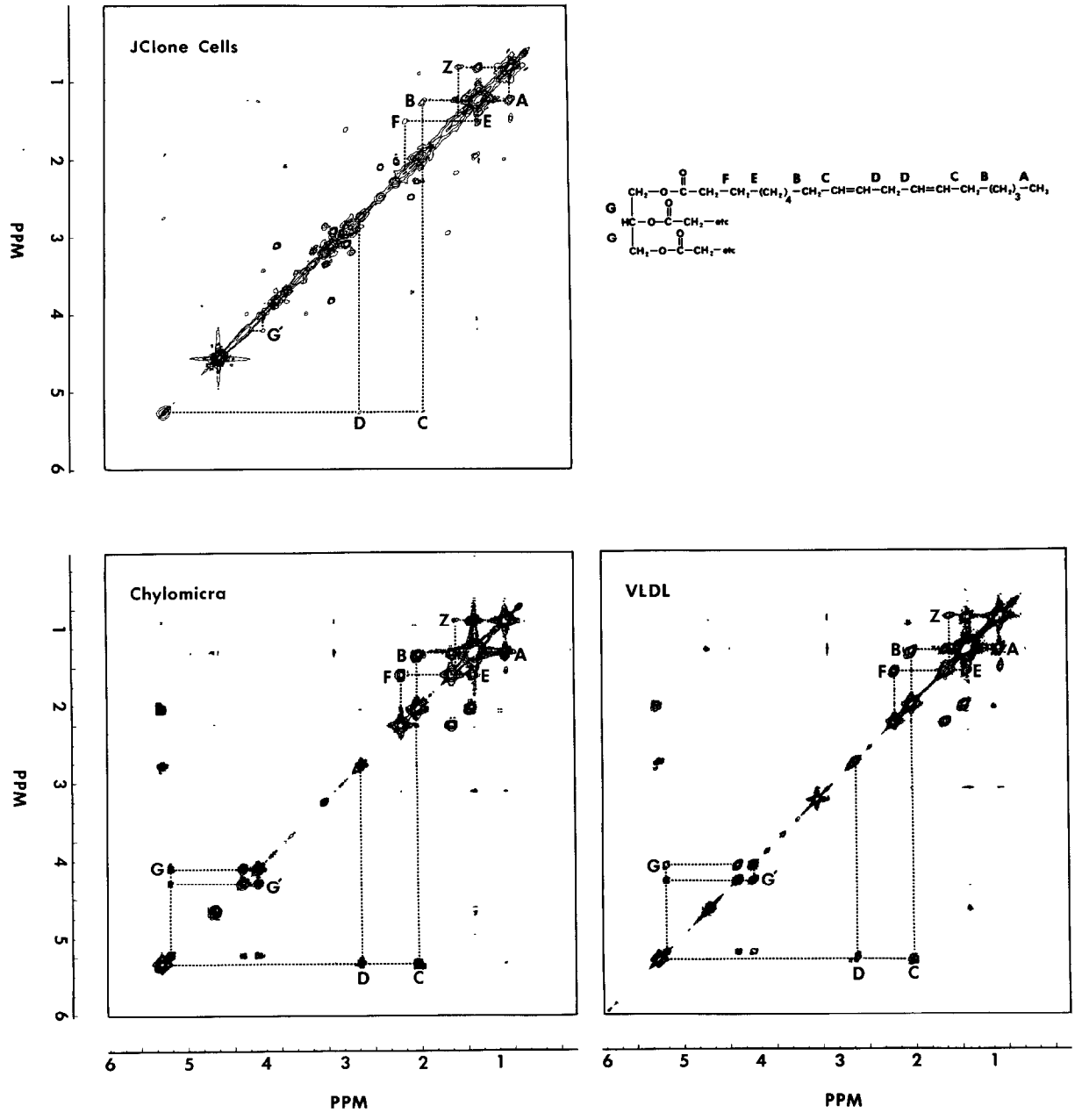

Fig.2. Symmetrised COSY spectra of chylomicra, VLDL and a suspension of J clone cells (1×10^8 cells) in phosphate-buffered saline in D_2O. Spectra were obtained at 37°C with the sample spinning and the residual HOD peak suppressed by gated irradiation. Lipid connectivities are indicated, and cross-peaks are designated according to the structure. Sine-bell and Gaussian (LB = −16, GB = 0.22) window functions were applied in the T_1 and T_2 domains, respectively, of the J clone spectrum, and sine-bell in both dimensions for the lipoprotein data. Z denotes the cross-peak linking the methyl and methine protons of the cholesterol alkyl chain.

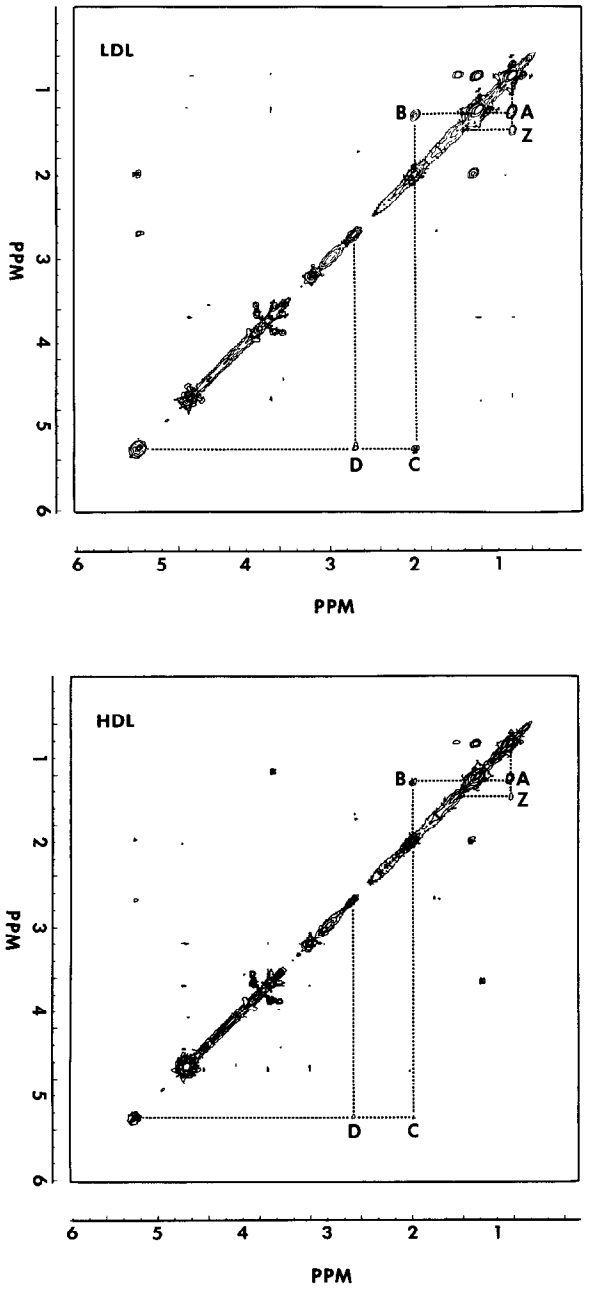

Fig.3. Symmetrised COSY spectra of LDL and HDL in phosphate-buffered saline in D_2O. Spectra were obtained at 37°C with the sample spinning and the residual HOD peak suppressed by gated irradiation. Lipid connectivities are indicated, and cross-peaks are designated according to the structure in fig.2. A sine-bell window function was applied in both the T_1 and T_2 domains.

suspension of rat mammary adenocarcinoma cells (J clone, fig.2). All the cross-peaks from the triglyceride molecule are present in the cells with the exception of G which is probably due to a lack of signal-to-noise. Cross-peak Z from the cholesterol alkyl chain is also visible in the cell spectrum. The remaining cross-peaks in the cell spectrum have been shown to arise from cytoplasmic components [4].

4. DISCUSSION

It was our intention to establish if any of the 4 main classes of lipoprotein resembled the triglyceride rich membrane domain found in the plasma membranes of cancer and other rapidly dividing cells [1]. Based on 2D NMR experiments, T_1 measurements and chemical analyses, both chylomicra and VLDL exhibit properties similar to the cancer cell membrane domain.

LDL and HDL have more phospholipid than triglyceride (table 1), with ratios of 2:1 and 10:1, respectively. Since phospholipid is a major constituent of the surface of all the lipoprotein particles, it is reasonable to assume that both lateral diffusion and exchange of lipid between particles could occur. However, in the case of the VLDL and chylomicra this diffusion is undetected since both particles are predominantly triglyceride. From these considerations the cell membrane domain appears more similar to VLDL and chylomicra than LDL or HDL, since no diffusive exchange of lipids has been observed in the cells and much of the ^1H NMR signal arises from neutral lipids. Until the cell membrane domain is isolated we are unable to establish its exact lipid and protein composition.

Another observation of particular interest to us was the absence of a long T_2 in any of the 4 classes of lipoproteins. Clearly the long T_2 measured for the triglycerides in the plasma membranes of cancer cells with the capacity to metastasize is due to a characteristic not present in lipoproteins from healthy donors.

It has been shown by others that many cancer patients have elevated levels of VLDL [18], and that most cancer cells have an increased number of LDL receptors on the cell surface which bind both LDL and VLDL [19]. Although a correlation between lipoprotein uptake and cancer was first postulated in the 1960's, only recently has the con-

nection between altered uptake and cellular behaviour patterns been made [20]. We do not yet know if the neutral lipids that give rise to the long T_2 values observed in cancer cell membranes are present in the lipoproteins of the cancer patient. Lipoproteins from the serum of patients with various types of cancer are now being investigated.

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