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The lipid fluidity of rat liver membrane subfractions

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1. The lipid fluidity of three major rat liver plasma-membrane subfractions, as well as Golgi apparatus and endocytic fractions, was assessed with a fatty acid spin probe by using e.s.r. techniques. 2. The sinusoidal (blood-facing) plasma-membrane subfraction was the most fluid of the three plasma-membrane regions. Fractions originating from the bile-canalicular and contiguous (lateral) regions were most rigid. Endocytic fractions isolated (endosomes and diacytosomes) were of a similar fluidity to fractions originating from the sinusoidal plasma-membrane region. By far the most fluid fractions examined were derived from the Golgi-apparatus complex. 3. The three plasma-membrane subfractions each showed a different response to the bilayer-fluidizing effect of benzyl alcohol. 4. Arrhenius-type plots of the order parameter S and outer hyperfine splitting, $2T_{\parallel}$, identified lipid-phase separations in the plasma-membrane subfractions.

The hepatocyte plasma membrane is a highly differentiated organelle showing extensive functional and structural heterogeneity (Motta *et al.*, 1978). The properties of the three major domains of the plasma membrane, i.e. the blood sinusoidal, lateral (contiguous) and bile canalicular, have been extensively documented by enzymic, chemical, receptor-binding and ultrastructural analysis of subcellular fractions originating predominantly from each of these domains (Wisher & Evans, 1975; Evans, 1980, 1981). The sinusoidal and canalicular plasma-membrane domains are highly dynamic, for they interact extensively with intracellular membranes during exocytosis. On the other hand, endocytic events appear to be confined mainly to the sinusoidal domain. Endocytic vesicular fractions have been prepared by following the receptor-mediated uptake by liver of circulating radioiodinated ligands, and these differ in properties from plasma-membrane and Golgi-apparatus subcellular fractions (Debanne *et al.*, 1982). We show here that there are major differences in lipid fluidity of these membrane compartments.

Methods

Preparation of liver membrane fractions

Plasma-membrane subfractions (zonal 'light', 'heavy' A, 'heavy' B and microsomal 'light') were prepared from liver homogenates of Sprague–Dawley rats (200–300g) as previously described (Wisher & Evans, 1975). Golgi-apparatus 'intermediate' and 'heavy' subfractions were prepared as described by Bergeron (1979). Diacytosome and endosome fractions were prepared from liver homogenates as described by Debanne *et al.* (1982).

To remove secretory components that may be associated with the isolated fractions, Golgi apparatus and sinusoidal plasma-membrane fractions were treated with 1M-Na_2CO_3 for 1h, pelleted by centrifugation and then resuspended (Fujiki *et al.*, 1982).

Spin labelling of liver membranes

The spin label I(12,3) was added to the liver membranes at 'low probe' concentrations as previously described in some detail (Whetton *et al.*, 1983*c*). E.s.r. measurements were made as previously described (Whetton *et al.*, 1983*c*) with a Varian E9-X band spectrometer. The polarity-corrected order parameter S used here was calculated by using the previously described equation (Gordon & Sauerheber, 1977).

Materials

The *N*-oxyl-4,4′-dimethyloxazolidine derivative of 5-oxostearic acid, I(12,3), was obtained from Syva Co., Palo Alto, CA, U.S.A. All other chemicals were from Sigma.

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Table 1. *$2T_{\parallel}$ and S values of I(12,3)-labelled liver membrane fractions*

Results are means ± S.D. for the numbers of experiments shown. Values were obtained at 30°C. The membrane subfractions were prepared as described in the Methods section.

Subfraction	Major subcellular origin of membranes	No. of expts.	$2T_{\parallel}$	S
Zonal 'heavy' B	Contiguous plasma membrane	9	52.7 ± 0.3	0.6645 ± 0.0057
Zonal 'heavy' A	Contiguous plasma membrane	6	52.1 ± 0.4	0.6598 ± 0.0057
Zonal 'light'	Canalicular plasma membrane	9	52.2 ± 0.4	0.6558 ± 0.0054
Microsomal 'light'	Sinusoidal plasma membrane	8	49.9 ± 0.2	0.6007 ± 0.0069
Golgi intermediate	Golgi secretory component	6	46.7 ± 0.7	0.5111 ± 0.0253
Golgi heavy	Golgi cisternal element	6	48.8 ± 0.8	0.5788 ± 0.0566
Endosomes	'Trafficking' vesicles	6	49.9 ± 0.2	0.6130 ± 0.0020
Diacytosomes	'Trafficking' vesicles	8	49.9 ± 0.3	0.6096 ± 0.0020

Table 2. *Effect of benzyl alcohol on I(12,3)-labelled liver plasma-membrane subfractions*

ΔS is defined as the percentage change (with respect to control membranes) in S induced by the addition of 50 mM-benzyl alcohol to the I(12,3)-labelled liver plasma-membrane fractions at 30°C. Results shown are means ± S.D. for six experiments on two membrane preparations.

Membrane fraction	Major subcellular origin of membranes	ΔS induced by 50 mM-benzyl alcohol
Zonal 'heavy' B	Contiguous plasma membrane	−4.24 ± 0.66
Zonal 'light'	Canalicular plasma membrane	−4.92 ± 0.25
Microsomal 'light'	Sinusoidal plasma membrane	−2.95 ± 0.29

Table 3. *Apparent temperatures of onset of discontinuities in the Arrhenius plots of I(12,3)-labelled liver plasma-membrane subfractions*

Both onset and end-point temperature are apparent on the Arrhenius plots for canalicular and sinusoidal membranes. However, the contiguous subfraction showed a single break. Results determined with zonal 'heavy' fraction A were similar to those with zonal 'heavy' fraction B. Results shown are means ± S.D. from four Arrhenius plots.

	Temp. (°C)	
	$2T_{\parallel}$	S
Zonal 'heavy' B (contiguous)	17.2 ± 1.7	16.4 ± 1.8
Zonal 'light' (canalicular)	17.8 ± 1.1	16.8 ± 1.7
	24.7 ± 0.1	23.6 ± 1.2
Microsomal 'light' (sinusoidal)	20.5 ± 1.8	20.0 ± 1.2
	29.0 ± 0.9	29.1 ± 1.4

Results

E.s.r. spectra were obtained with membrane fractions labelled at experimentally determined 'low probe' concentrations of the I(12,3) spin label; under these conditions probe–probe interactions are negligible (Sauerheber *et al.*, 1977; Gordon *et al.*, 1980). Table 1 shows the order parameter S and $2T_{\parallel}$ values of the various I(12,3)-labelled liver membrane fractions examined. The plasma-membrane fractions originating from the lateral and canalicular plasma-membrane regions have a significantly higher $2T_{\parallel}$ and order-parameter values and hence greater rigidity than the blood-sinusoidal fraction. The endocytic endosome and diacytosome fractions were both of a similar fluidity. The two Golgi-apparatus fractions were the most fluid membrane fractions examined. Since the fluidity parameters measured may be influenced by free lipids encased inside membrane vesicles, and since Golgi fractions are known to contain lipoprotein destined for secretion into the blood sinusoids, these fractions were subjected to additional treatments that release non-membranous components (Fujiki *et al.*, 1982). However, extraction of Golgi or sinusoidal plasma-membrane fractions with 1 M-Na_2CO_3, pH 10, failed to change the inner and outer hyperfine splitting values measured (Table 1).

The effects of the bilayer-fluidizing agent benzyl alcohol (50 mM) on the order parameter S of the plasma-membrane fractions were investigated. A greater increase in fluidity (decrease in S) was elicited by benzyl alcohol in the more-rigid plasma-membrane subfractions (canalicular and contiguous) than was observed with the more-fluid sinusoidal membrane subfraction (Table 2).

The temperature-dependences of the order parameter S and the outer hyperfine splitting $2T_{\parallel}$ (Fig. 1) of I(12,3)-labelled liver plasma-membrane subfractions were also investigated. These exhibited discontinuities at distinct temperatures, shown in Table 3. Different values of $2T_{\parallel}$ and S were observed with the various membrane preparations over the temperature range used (Fig. 1).

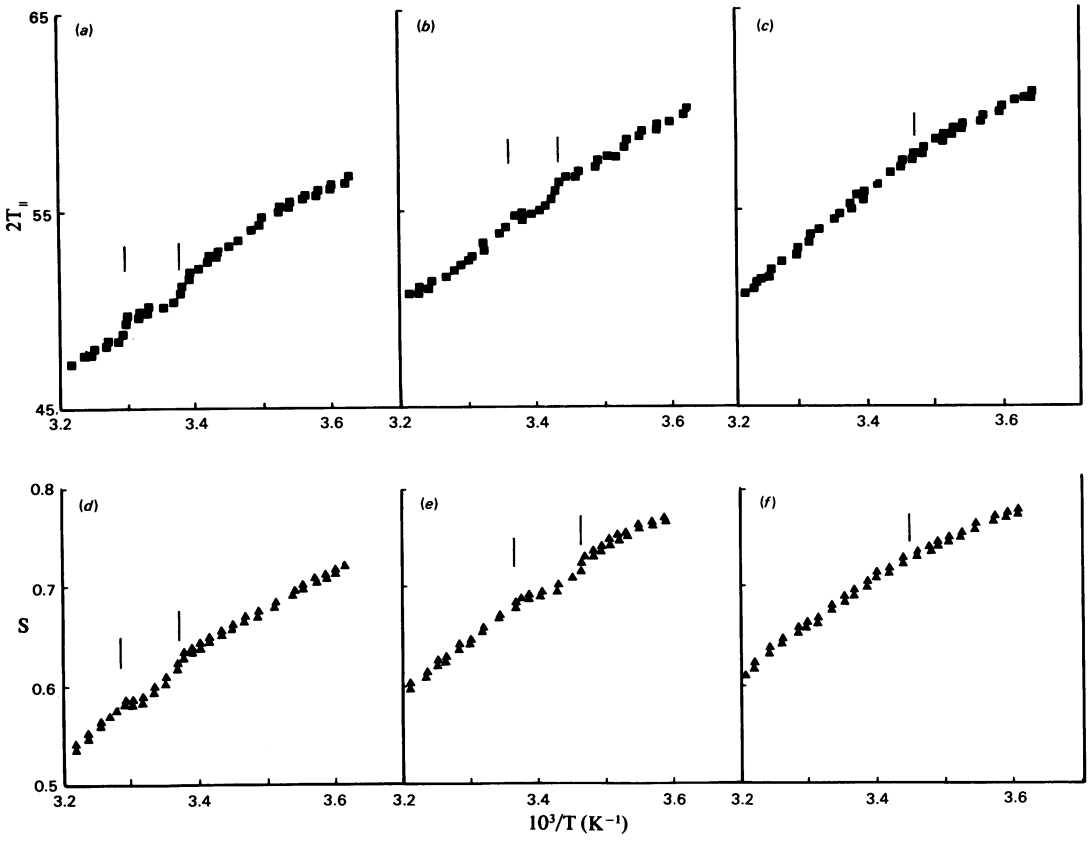

Fig. 1. *Arrhenius-type plots of $2T_{\parallel}$ (a–c) and order-parameter S (d–f) values for I(12,3)-labelled plasma-membrane subfractions: (a), (d), sinusoidal; (b), (e), canalicular; (c), (f), contiguous*

Vertical lines represent the onset of discontinuities in the Arrhenius-type plots. For explanation of fraction nomenclature, see the Methods section and Table 1.

Discussion

The results presented show, by using spin-labelled membranes, that large differences exist in the lipid fluidity of subcellular membranes originating from different regions of the hepatocyte's cell surface. Furthermore, membranes derived from intracellular regions that are in functional continuity with specific regions of the plasma membrane during endocytosis (the endosome and diacytosome fractions) and exocytosis (Golgi subfractions) are more fluid than the averaged fluidity of plasma-membrane fractions.

The membrane fractions can be placed in order of their increasing membrane fluidity (Table 1): contiguous fraction < canalicular < sinusoidal < Golgi membrane fractions. The cholesterol and sphingomyelin contents of these membranes also decrease in a parallel fashion (Zambrano *et al.*, 1975; Kremmer *et al.*, 1976). It may be that these differences in fluidity are in part a consequence of the very different cholesterol and sphingomyelin concentrations present, for increasing cholesterol concentrations cause a decrease in lipid fluidity (Chapman, 1975). Also, sphingomyelin will cause a decrease in rat liver plasma-membrane fluidity, as its associated fatty acyl chains are unusually long and fully saturated (Van Hoeven *et al.*, 1979). The difference observed in the lipid fluidity of the plasma-membrane subfractions extended over the whole temperature range tested (Fig. 1). However, the form of Arrhenius-type plots of S and $2T_{\parallel}$ differed for the various plasma-membrane subfractions, with distinct discontinuities appearing at different temperatures (Table 3). These discontinuities have previously been attributed to the onset of lipid-phase separations within the lipid bilayer (Houslay *et al.*, 1976; Gordon *et al.*, 1980; Whetton *et al.*, 1982) and appear to exert a marked effect on

the activities of a wide variety of integral plasma-membrane enzymes, including 5'-nucleotidase, adenylate cyclase, Na^+- and K^+-stimulated ATPase, alkaline phosphodiesterase and cyclic AMP phosphodiesterase (Gordon *et al.*, 1980; Dipple *et al.*, 1982; Whetton *et al.*, 1982). The discontinuities observed seem to undergo a progressive loss of definition from sinusoidal to canalicular to contiguous plasma-membrane subfractions, which may be a consequence of the parallel increase in cholesterol concentrations present in these subfractions. Indeed, elevated cholesterol concentrations have been shown to smear or abolish lipid-phase separations and transitions in both model membranes (Lee, 1975) and a liver plasma-membrane fraction (Whetton *et al.*, 1983*a*). Elsewhere (Gordon *et al.*, 1980; Whetton *et al.*, 1982, 1983*a*,*b*) we have presented evidence that such a lipid-phase separation occurs in 'cholesterol-poor' regions of the outer half of the bilayer of liver plasma membranes.

The response of liver plasma-membrane fractions to the bilayer-fluidizing agent benzyl alcohol also highlighted differences between these three subfractions examined (Table 2). The more fluid (sinusoidal) membrane region was fluidized to a lesser extent by benzyl alcohol than were the canalicular or contiguous membrane preparations. This suggests that the response of these subfractions to agents capable of modulating membrane fluidity is a function of their lipid composition. The marked difference in lipid fluidity of the plasma-membrane subfractions may be of importance to their function. The bile-canalicular membrane must possess sufficient fluidity to function as an exocytic interface, and to maintain at the same time a barrier function to limit its dissolution by the action of bile contained within the biliary spaces. The lateral (or contiguous) plasma-membrane region features extensively in facilitating cell–cell interactions and in controlling the paracellular pathway of transport of low-molecular-weight solutes from blood to bile. The higher protein/lipid ratio and low fluidity of this region may reflect a requirement for mechanical rigidity of the surface region containing intercellular junctions linking contiguous hepatocytes. The higher lipid fluidity of the sinusoidal plasma-membrane fractions is in accord with the highly dynamic nature of this cell-surface region. This membrane surface interacts with the highly fluid endosomes and diacytosomes that transport ligands to intracellular destinations (Debanne *et al.*, 1982). Our results suggest that the high lipid fluidity of the various subfractions derived from intracellular membranes that have functional interactions with the sinusoidal plasma-membrane region may be of general importance in the maintenance of organelle integrity during membrane recycling processes (Morré, 1977).

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