Preferential localization of rat liver D-*myo*-inositol 1,4,5-trisphosphate/1,3,4,5-tetrakisphosphate 5-phosphatase in bile-canalicular plasma membrane and ‘late’ endosomal vesicles

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Previous studies have shown that most of the inositol 1,4,5-trisphosphate/inositol 1,3,4,5-tetrakisphosphate 5-phosphatase activity of rat hepatocytes is associated with the plasma membrane [Shears, Parry, Tang, Irvine, Michell & Kirk (1987) *Biochem. J.* **246**, 139–147]. We now show that the specific activity of this enzyme is highest in the bile-canalicular domain of the plasma membrane, at the opposite pole of the hepatocyte from the presumed site of receptor-mediated formation of inositol 1,4,5-trisphosphate. In intact hepatocytes and in sealed membrane vesicles originating from the bile-canalicular domain of the plasma membrane, the 5-phosphatase activity was mostly latent and therefore located at the cytoplasmic surface. A substantial amount of 5-phosphatase was also found in rat liver endosomal fractions, particularly a ‘late’ endosomal subfraction, indicating that this enzyme may be transported between the sinusoidal plasma membrane and other cellular membranes.

INTRODUCTION

Receptor-activated hydrolysis of PtdIns(4,5) P_2 yields Ins(1,4,5) P_3 , some of which is converted by kinase action into Ins(1,3,4,5) P_4 . These two inositol phosphates probably act in concert as intracellular messengers, bringing about a sustained rise in cytosolic $[Ca^{2+}]$ (reviewed by Downes & Michell, 1985; Berridge, 1987; Irvine *et al.*, 1988). Both Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 are inactivated by specific 5-phosphate phosphatase action (Connolly *et al.*, 1987; Shears *et al.*, 1987*a,b*).

In liver, the Ins(1,4,5) P_3 /Ins(1,3,4,5) P_4 5-phosphatase is associated with the plasma membrane (Storey *et al.*, 1984; Seyfred *et al.*, 1984; Joseph & Williams, 1985; Shears *et al.*, 1987*b*). In many cells, notably epithelia, the plasma membrane is sharply demarcated by tight junctions into separate functional domains (Simmons & Fuller, 1985). For example, the main site of hormone action on liver cells is the sinusoidal plasma-membrane surface that is bathed by the blood, whereas bile is secreted by the same cells across a different plasma-membrane region that forms the boundary of the bile canaliculus. Plasma membranes can be isolated as vesicles from these two surfaces of the cell, and also from the ‘contiguous’ areas that are laterally apposed in adjacent cells (Wisher & Evans, 1975; Evans, 1980). The sinusoidal plasma-membrane domain is in a dynamic state, being the major site of constant endocytic trafficking. The endocytic process involves a series of intracellular tubulo-vesicular compartments which are implicated in the continuous cycling of components between the cell surface and endomembrane systems. In liver the

endosomal apparatus is also involved in the net transcellular movement of certain molecules (e.g. IgA and its receptor) from the sinusoidal face of the cell to the bile canaliculus (Goldstein *et al.*, 1985; Mellman *et al.*, 1987). Endosomal vesicles have been isolated from several parts of the endosomal apparatus of liver (Evans & Flint, 1985; Mullock *et al.*, 1987; Belcher *et al.*, 1987).

We have previously demonstrated that the Ins(1,4,5)- P_3 /Ins(1,3,4,5) P_4 5-phosphatase of liver is recovered in membranes that are highly enriched in phosphodiesterase I and leucine β -naphthylamidase (Storey *et al.*, 1984; Shears *et al.*, 1987*a,b*), which are both marker enzymes for plasma membranes (Evans, 1987). We have now undertaken further experiments to determine in which domain or domains of the hepatocyte plasma membrane the 5-phosphatase is localized and whether it is present in endocytic vesicles.

MATERIALS AND METHODS

Materials

[4,5- ^{32}P]Ins(1,4,5) P_3 was prepared as described by Rubiera *et al.* (1988), except that, after its second elution from an ion-exchange column in 10 ml of 0.8 M-ammonium formate/0.1 M-formic acid, the preparation was diluted 5-fold and applied to a 0.1 ml Bio-Rad ion-exchange column (AG1X8; 200–400 mesh; formate form). The formate ions were eluted with 5×0.5 ml of 0.1 M-NH $_4$ HCO $_3$, and then the [4,5- ^{32}P]Ins(1,4,5) P_3 was eluted (83 % recovery) with 5×0.5 ml of 0.5 M-NH $_4$ HCO $_3$ (freshly made, filtered and prewarmed

Abbreviations used: Ins(1,4) P_2 , Ins(1,4,5) P_3 , Ins(1,3,4) P_3 and Ins(1,3,4,5) P_4 , D-*myo*-inositol bis-, tris- and tetrakis-phosphates, with locants designated where appropriate; PtdIns(4,5) P_2 , phosphatidylinositol 4,5-bisphosphate.

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to 45 °C immediately before use). The NH_4HCO_3 was removed by freeze-drying. The distribution of the ^{32}P between the 4- and 5-phosphates (typically 1:4) was ascertained by using erythrocyte ghosts (Downes *et al.*, 1982; Shears *et al.*, 1987a).

[4,5- ^{32}P]Ins(1,3,4,5) P_4 was prepared by incubating a 2% (w/v) liver supernatant (see Shears *et al.*, 1987a) with 0.2 μCi of [4,5- ^{32}P]Ins(1,4,5) P_3 in 1 ml of medium containing 40 mM-glycine (pH 9.0 with KOH), 20 mM-ATP and 20 mM-MgSO₄. After 30 min at 37 °C, approx. 70% of the [4,5- ^{32}P]Ins(1,4,5) P_3 was converted into [4,5- ^{32}P]Ins(1,3,4,5) P_4 . The reactions were quenched and neutralized, then the [4,5- ^{32}P]Ins(1,3,4,5) P_4 was twice chromatographed on a Bio-Rad column (Shears *et al.*, 1987a). The preparation was then desalted as described above and an 87% recovery was obtained.

[^3H]Ins(1,4,5) P_3 was purchased from Amersham International, and [^3H]Ins(1,3,4,5) P_4 was purchased from New England Nuclear. The collagenase for the hepatocyte preparations was purchased from Boehringer. Non-radioactive Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 were purchased from Calbiochem. For other sources of chemicals see Shears *et al.* (1987a,b).

Preparation of hepatocytes and subcellular fractions

Hepatocytes were prepared as described by Shears *et al.* (1987a), except that the final preparation was washed and suspended in the 'intracellular' medium used to assay inositol phosphate metabolism (see below). The cells were stored on ice and used within 20 min. Immediately after isolation, $90 \pm 2\%$ ($n = 4$) of total lactate dehydrogenase was intracellular, and this decreased to $79 \pm 2\%$ ($n = 4$) after the cells had been stored and then incubated at 37 °C for 6 min.

Liver plasma-membrane fractions originating from the sinusoidal, lateral and canalicular domains of the cell were prepared as described by Wisher & Evans (1975) as modified by Evans *et al.* (1980). Endosomal fractions, prepared as previously described (Evans & Flint, 1985; Saermark *et al.*, 1985), were collected from a sucrose gradient and subfractionated by equilibrium centrifugation in shallow Nycodenz gradients. The resultant vesicular membrane fractions were designated as follows: DN-2 or 'early' endosomes accumulate various internalized ligands 2–5 min after uptake by the liver, and DN-1 or 'late' endosomes accumulate these ligands after 15 min. A further fraction, enriched in binding sites for insulin and asialoglycoprotein receptors and designated D-R, was collected from the interface of the Nycodenz gradient and the sucrose cushion.

These subcellular fractions were stored at -20 °C before use, except when enzyme latencies were determined, whereupon they were stored on ice and used within 6 h of their preparation.

Assay of Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 5-phosphatase

The incubation medium (0.2 ml) was of approximately physiological ionic strength and pH (Shears *et al.*, 1987b), and also included 1 mM-dithiothreitol and 0.5% (w/v) bovine serum albumin. Saponin was omitted from some incubations where indicated. The 5-phosphatase activity was usually assayed against either 15 μM -Ins(1,4,5) P_3 {+ 12000 d.p.m. of [^3H]Ins(1,4,5) P_3 and, where indicated, 6000 d.p.m. of [4,5- ^{32}P]Ins(1,4,5) P_3 }, or 1 μM -Ins(1,3,4,5) P_4 {+ 12000 d.p.m. of [^3H]Ins(1,3,4,5) P_4 and, where indicated, 6000 d.p.m. of [4,5- ^{32}P]Ins(1,3,4,5) P_4 }. In

order to determine the kinetic parameters of the 5-phosphatase, incubations were performed with either 80, 50, 25, 15, 10, 5 and 2.5 μM -Ins(1,4,5) P_3 , or 10, 5, 2.5, 1, 0.5 and 0.25 μM -Ins(1,3,4,5) P_4 . V_{max} and K_m values were obtained from computer-fitted Eadie-Hofstee plots. All incubations, in quadruplicate, were at 37 °C and initiated with 0.02 ml of an appropriately diluted tissue sample. Preliminary experiments indicated that reaction rates were linear at the concentrations of protein used in the assays, provided that less than 25% of the substrate was metabolized.

After 6 min the incubations were quenched with 0.08 ml of 2 M-HClO₄ and placed on ice. The perchlorate-precipitated protein was removed by centrifugation for 5 min at 12000 g, then 265 μl of each supernatant was neutralized and loaded on to a Bio-Rad ion-exchange column (AG1X8; 200–400 mesh; formate form) as described by Shears *et al.* (1987a). [^{32}P]P_i was eluted with 10 ml of 180 mM-ammonium formate. For the Ins(1,4,5)- P_3 5-phosphatase assays, the other products (which were only Ins P_2) were eluted with 10 ml of 0.4 M-ammonium formate/0.1 M-formic acid, and then the Ins(1,4,5) P_3 was eluted in 10 ml of 0.8 M-ammonium formate/0.1 M-formic acid. For the Ins(1,3,4,5) P_4 5-phosphatase assays, the other products (which were only Ins P_3) were eluted with 8–12 ml of 0.8 M-ammonium formate/0.1 M-formic acid (the precise volume depended on the elution characteristics of the particular batch of resin, as determined with radiolabelled standards). The Ins(1,3,4,5) P_4 was then eluted with 10 ml of 1.2 M-ammonium formate/0.1 M-formic acid. Samples (2.5 ml) of each eluate were assayed by liquid-scintillation spectrometry (Shears *et al.*, 1987a).

Subcellular-marker assays

Phosphodiesterase I (EC 3.1.4.1) was assayed as described by Razzel (1963), except that the assay medium was that used to study inositol phosphate metabolism (see above). Lactate dehydrogenase (EC 1.1.1.27) was assayed as described by Bergmeyer & Bernt (1974). 5'-Nucleotidase (EC 3.1.3.5) was assayed as described by Michell & Hawthorne (1965). Leucine β -naphthylamidase (EC 3.4.11.1) was assayed as described by Goldberg & Rutenburg (1958). Asialoglycoprotein-binding protein was determined by using asialo-orosomucoid as the ligand (Debanne *et al.*, 1982). Protein was assayed as described by Bradford (1976), with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The Ins(1,4,5) P_3 /Ins(1,3,4,5) P_4 phosphatase activity of liver plasma membrane and endosomal subfractions removes the 5-phosphate group

In previous studies we used ^3H - and ^{32}P -labelled substrates to demonstrate that liver homogenates and plasma membranes removed only the 5-phosphate from both Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 (Storey *et al.*, 1984; Shears *et al.*, 1987a,b). This conclusion was confirmed with each of the separated plasma-membrane subfractions and the 'late' endosome fractions by assays in which the membranes were allowed to hydrolyse either (a) a mixture of [^3H]Ins(1,4,5) P_3 and [4,5- ^{32}P]Ins(1,4,5)- P_3 , or (b) a mixture of [^3H]Ins(1,3,4,5) P_4 and [4,5- ^{32}P]Ins(1,3,4,5) P_4 . In both cases most of the ^{32}P was in the

5-position (see the Materials and methods section). In all experiments, the $^3\text{H}:^{32}\text{P}$ ratio of the InsP_2 or InsP_3 produced was that expected for attack solely on the 5-phosphate of either $\text{Ins}(1,4,5)\text{P}_3$ or $\text{Ins}(1,3,4,5)\text{P}_4$ (results not shown).

$\text{Ins}(1,4,5)\text{P}_3/\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase activity is concentrated in the bile-canalicular domain of the hepatocyte plasma membrane

Wisher & Evans (1975) showed that liver plasma-membrane preparations can be further fractionated, yielding three subfractions that represent substantially purified preparations of the sinusoidal, lateral and bile-canalicular regions of the liver cell surface. We performed several experiments which confirmed that the purity of the preparations was similar to that reported previously (Table 1): the specific activities of 5'-nucleotidase, phosphodiesterase I and leucine β -naphthylamidase were enriched 46–94-fold relative to homogenates, and were 4–10-fold greater in the bile-canalicular fractions compared with specific activities in the sinusoidal and lateral fractions. Our data also confirmed that cell-surface receptors were most abundant

in the sinusoidal membrane fraction, where there was a 2.5- and 8-fold greater binding capacity for asialo-orosomucoid compared with lateral and bile-canalicular fractions respectively (Table 1).

The distribution of 5-phosphatase activity in the various plasma-membrane fractions, measured with either $\text{Ins}(1,4,5)\text{P}_3$ or $\text{Ins}(1,3,4,5)\text{P}_4$, closely followed the distribution of markers for the bile-canalicular membranes (Tables 1 and 2). For both 5-phosphatase activities, the bile-canalicular membranes showed a 4-fold greater activity than the sinusoidal membranes.

In designing the 5-phosphatase assays, we elected to measure activity at approximately the K_m for each inositol polyphosphate, in order to conserve substrate. This assay left open the possibility that the apparent differences between the domains might arise from differences in enzyme properties rather than enzyme amounts. To check this, simple kinetic constants were determined for the three plasma-membrane subfractions, and for the 'late' endosomal subfraction. All fractions exhibited the same K_m values [approx. $15\ \mu\text{M}$ for $\text{Ins}(1,4,5)\text{P}_3$ and $1.2\ \mu\text{M}$ for $\text{Ins}(1,3,4,5)\text{P}_4$; Table 2] and, as expected, the $V_{\max}$ value for each membrane

Table 1. Distribution of subcellular markers in preparations of sinusoidal, lateral and bile-canalicular regions of plasma membranes

Assays were performed as described in the Materials and methods section. Data are means $\pm$ S.E.M. with the numbers of determinations in parentheses; n.d., not determined.

Fraction	Marker activity ($\mu\text{mol/h}$ per mg of protein)			Asialo-orosomucoid binding (ng/mg of protein)
	5'-Nucleotidase	Leucine β -naphthylamidase	Phosphodiesterase I	
Homogenate	1.6 (2)	0.3 (2)	10 ± 3 (4)	n.d.
Plasma membranes				
Sinusoidal	37 ± 2.5 (3)	2.3 (2)	75 ± 19 (3)	9.8 ± 1.4 (3)
Lateral	53 ± 13 (3)	2.2 (2)	163 ± 70 (3)	3.8 ± 0.5 (3)
Canalicular	151 ± 45 (3)	13.7 (2)	729 ± 114 (4)	1.2 ± 0.6 (3)

Table 2. Distribution and K_m of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatases, in plasma-membrane domains and endosomal subfractions prepared from liver

All assays were performed as described in the Materials and methods section. Data are means $\pm$ S.E.M. where appropriate, with the numbers of determinations in parentheses. The data for phosphodiesterase I include those listed in Table 1.

Fraction	$\text{Ins}(1,4,5)\text{P}_3$ phosphatase		$\text{Ins}(1,3,4,5)\text{P}_4$ phosphatase		Phosphodiesterase I
	Sp. activity (nmol/min per mg)	K_m (μM)	Sp. activity (nmol/min per mg)	K_m (μM)	
Homogenate	3 ± 0.4 (6)	n.d.	0.2 ± 0.05 (3)	n.d.	10 ± 3 (3)
Plasma membranes					
Sinusoidal	35 ± 2 (4)	15 ± 3 (3)	2.5 ± 0.4 (3)	1.3 (2)	75 ± 19 (3)
Lateral	49 ± 18 (4)	12 (1)	3.4 ± 0.9 (3)	1.7 (1)	163 ± 70 (3)
Canalicular	140 ± 5 (4)	15 (2)	10.3 ± 1.2 (5)	0.9 ± 0.3 (3)	729 ± 114 (4)
Endosomes					
D-R	8 ± 1 (4)	n.d.	0.8 (2)	n.d.	23 ± 4 (3)
'Early'	22 ± 5 (4)	n.d.	2.2 (2)	n.d.	110 ± 4 (3)
'Late'	71 ± 9 (4)	18 (1)	5.1 ± 0.9 (2)	1.1 (1)	135 ± 23 (3)

preparation was approximately double the rate of substrate hydrolysis observed under the assay conditions which we routinely employed (results not shown). The $V_{\max}$ for Ins(1,4,5) P_3 was 10–15 times greater than for Ins(1,3,4,5) P_4 (results not shown). Thus it appears likely that each membrane preparation contains the same species of Ins(1,4,5) P_3 /Ins(1,3,4,5) P_4 5-phosphatase, but that this enzyme is present at a much higher concentration in the bile-canalicular domain of the plasma membrane.

Our value for the K_m for Ins(1,4,5) P_3 5-phosphatase (15 μM , Table 1) is much higher than the value of 1 μM reported in earlier studies with liver (Joseph & Williams, 1985); the reason for this discrepancy is not known. On the other hand, our K_m values for Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 (Table 1) are within the range of values [3–25 μM for Ins(1,4,5) P_3 , 0.8–3 μM for Ins(1,3,4,5) P_4] that have been reported for the membrane-bound 5-phosphatase in human erythrocytes (Downes *et al.*, 1982), pancreatic islets (Rana *et al.*, 1986) and brain (Hansen *et al.*, 1987), and also the soluble 5-phosphatase from platelets (Connolly *et al.*, 1987) and the 'Type 1' soluble 5-phosphatase from brain (Hansen *et al.*, 1987). On the other hand, a 'Type 2' phosphatase from brain cytosol has a very low affinity for Ins(1,3,4,5) P_4 (> 150 μM ; Hansen *et al.*, 1987), and seems very likely to be a different subspecies of enzyme.

Bile-canalicular 5-phosphatase is located at the cytoplasmic surface of the membrane

Previous work using resealed human erythrocyte ghosts demonstrated that the active site of Ins(1,4,5) P_3 5-phosphatase is located at the cytoplasmic surface of the red-cell plasma membrane (Shears *et al.*, 1987a). However, it cannot be taken for granted that other cell types do not have the capacity to metabolize Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 at the outer aspect of the cell surface. For example, intestinal-epithelial cells dephosphorylate these compounds by using extracellular phosphatases (Rubiera *et al.*, 1988).

We first examined the topographical location of the 5-phosphatase on liver membranes by comparing the rates of hydrolysis of Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 by intact and saponin-permeabilized hepatocytes. When hepatocytes were incubated in the 'intracellular' medium in the absence of saponin, approx. 20% of hepatocytes were permeable to lactate dehydrogenase (Table 3). This leakiness presumably accounts for the basal rate of dephosphorylation of Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 . The addition of saponin, which released all lactate dehydrogenase (results not shown), caused a 4-fold stimulation of the rate of hydrolysis of both polyphosphates (Table 3). These data indicate that hepatocytes possess predominantly intracellular 5-phosphatase activity.

The topographical distribution of the enzyme was also studied in isolated membrane vesicles. Since phosphodiesterase I activity is located on the exterior of the hepatocyte plasma membrane (Evans, 1974), we compared the structure-linked latencies of phosphodiesterase I with that of the 5-phosphatase. Bile-canalicular vesicles have been shown to be intact and right-side-out (Inoue *et al.*, 1983; Meier *et al.*, 1984) when prepared by procedures that are similar to those developed by Evans (1970) and Wisher & Evans (1975). This observation was confirmed in our preparations of canalicular vesicles: in the absence of detergent there was full activity of phosphodiesterase I, indicating that this ectoenzyme was in the same orientation as in the intact cell, but only 20% of the total 5-phosphatase activity was expressed, indicating that this enzyme was largely intravesicular (Table 3).

The other liver plasma-membrane subfractions consist of morphologically more diverse membrane vesicles and fragments (Wisher & Evans, 1975), and the results were less clear-cut. In the sinusoidal-plasma-membrane subfraction, about one-third of the phosphodiesterase I was latent (Table 3). The 5-phosphatase activity of this preparation showed structure-linked latency of about

Table 3. Latency of Ins(1,4,5) P_3 /Ins(1,3,4,5) P_4 5-phosphatase in hepatocytes, plasma-membrane domains and endosomal subfractions prepared from liver; comparison with phosphodiesterase I and lactate dehydrogenase

Latencies were determined by incubating the preparations, as described in the Materials and methods section, in the presence and absence of saponin. The data on hepatocytes are from three preparations, each incubated at a concentration of approx. 4 mg dry wt./ml. Ins(1,4,5) P_3 5-phosphatase and phosphodiesterase I activities in subcellular fractions were determined in two preparations, one of which was also assayed for Ins(1,3,4,5) P_4 5-phosphatase. Data are means $\pm$ S.E.M. where appropriate. Where duplicates were obtained, their individual values were never more than 6 percentage units from the mean, except for (a) phosphodiesterase latency in the sinusoidal membranes (duplicates were 50% and 26%) and (b) Ins(1,4,5) P_3 5-phosphatase latency in lateral membranes (duplicates were 52% and 31%). Abbreviation: n.d. = not determined.

Preparation	Latency (%)			
	Ins(1,4,5) P_3 phosphatase	Ins(1,3,4,5) P_4 phosphatase	Phospho- diesterase I	Lactate dehydrogenase
Hepatocytes	77 $\pm$ 2	73 $\pm$ 3	n.d.	79 $\pm$ 3
Plasma membranes				
Sinusoidal	70	70	38	—
Lateral	42	36	1	—
Canalicular	77	82	0	—
Endosomes				
D-R	63	58	41	—
'Early'	58	52	72	—
'Late'	78	93	59	—

two-thirds of the total activity against either $\text{Ins}(1,4,5)P_3$ or $\text{Ins}(1,3,4,5)P_4$ (Table 3). The most likely explanation of these results is that about two-thirds of these vesicles were oriented right-side-out, and contained intravesicular 5-phosphatase activity. The remaining one-third of these vesicles may be sealed plasma-membrane fragments of reversed orientation and/or contaminating vesicles derived from the endosomal compartment (see below).

The phosphodiesterase I activity in the lateral membrane subfraction showed no latency, and only one-third to one-half of the 5-phosphatase was latent. These results suggest that this fraction is a mixture of (a) a majority of membrane fragments or sheets with free access to substrates at both membrane surfaces (as indicated by electron microscopy; Wisher & Evans, 1975), and (b) a minority of sealed vesicles of normal orientation.

All of the data in Table 3 support the conclusion that the $\text{Ins}(1,4,5)P_3/\text{Ins}(1,3,4,5)P_4$ 5-phosphatase of hepatocytes is concentrated at the cytoplasmic face of the bile-canalicular domain of the plasma membrane.

$\text{Ins}(1,4,5)P_3/\text{Ins}(1,3,4,5)P_4$ 5-phosphatase in endosome-enriched fractions

Two isolated subfractions of the endosomal system, designated DN-2 and DN-1, possess characteristics indicating that they are derived from the compartment involved in membrane trafficking at the 'early' and 'late' stages respectively. A third subfraction, D-R, may be

involved in retroendocytosis of components to the sinusoidal plasma membrane (see Evans & Flint, 1985). All of these fractions contain some phosphodiesterase I, but even in 'late' endosomal fractions, where specific activity of this enzyme was highest, this was only about one-fifth the specific activity of the bile-canalicular plasma-membrane fraction (Table 2). About two-thirds of the phosphodiesterase I of the 'late' endosomal fraction was latent, in contrast with the entirely non-latent behaviour of this enzyme in bile-canalicular membrane vesicles (Table 3). These data indicate that the majority of the phosphodiesterase I of the 'late' fraction is located on the inner surface of the endocytic membranes *in vivo*. Such an orientation is that expected if the endosomal compartment is involved in intracellular trafficking of phosphodiesterase I (Quintart *et al.*, 1984). The 'early' and D-R endosomal subfractions contained smaller specific activities of phosphodiesterase I, about half of which was latent (Table 3).

The $\text{Ins}(1,4,5)P_3/\text{Ins}(1,3,4,5)P_4$ 5-phosphatase activity of the 'late' endosomal fractions was about half that of the bile-canalicular membrane fractions. From the above consideration of the phosphodiesterase I content and latency of 'late' endosomal subfractions, it appears that contamination with plasma-membrane fragments can only have accounted for a small portion of this endosomal 5-phosphatase. Activity in the other endosome-enriched fractions was lower, but even in D-R, the least active fraction, the 5-phosphatase specific activity was about

Fig. 1. Distribution and apparent topography of phosphodiesterase I and $\text{Ins}(1,4,5)P_3/\text{Ins}(1,3,4,5)P_4$ 5-phosphatase in hepatocytes

The diagram shows the three domains of the liver plasma membrane and the early and late endosomal compartments that may provide a transcellular vesicular pathway connecting the sinusoidal and canalicular domains. $\text{Ins}(1,4,5)P_3$ and $\text{Ins}(1,3,4,5)P_4$ may be preferentially hydrolysed at the bile canaliculus. See the text for further details. Key: $\blacktriangle$, $\text{Ins}(1,4,5)P_3/\text{Ins}(1,3,4,5)P_4$ 5-phosphatase; $\bullet$, phosphodiesterase I.

3 times that of the homogenate. Since $\text{Ins}(1,4,5)\text{P}_3/\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase was located on the inner face of plasma membrane vesicles (see above), we expected it to show the opposite behaviour in endosomal vesicles. On the contrary, the $\text{Ins}(1,4,5)\text{P}_3/\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase activity of the 'late' endosomal sub-fraction was highly latent (Table 3). One possible explanation for these data may be that 'late' endosomal fractions contain a number of multivesicular bodies (see, e.g., Wileman *et al.*, 1985, for a review). The phosphodiesterase I is presumably on the inner surface of the outer membrane of the multivesicular body, therefore accounting for its latency in these fractions (Table 3). The latency of the $\text{Ins}(1,4,5)\text{P}_3/\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase can be accounted for if the enzyme were on the outer surface of the small vesicles inside the multivesicular body. These topographical considerations (Fig. 1) also provide an explanation for the location of the 5-phosphatase and the alkaline phosphodiesterase on opposite sides of the membrane of the bile canaliculus.

Implications of the bile-canalicular localization and possible intracellular trafficking of $\text{Ins}(1,4,5)\text{P}_3/\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase

In Fig. 1 we postulate that the 5-phosphatase is transferred from the sinusoidal to the canalicular plasma-membrane domains via an endocytic pathway. This proposal is based on current views of the biogenesis of liver plasma-membrane domains, from experiments with fucoproteins (Evans *et al.*, 1980), or more comprehensively with various antigens (Bartles *et al.*, 1987). These studies support the view that many integral membrane proteins travel from the sinusoidal to the canalicular domain via an intracellular membrane-connecting system such as the endocytic network. The passage of polymeric IgA-receptor complexes from the sinusoidal to the canalicular space appears to follow the same transcellular pathway (Mostov & Simister, 1985). An alternative possibility is that there is a direct insertion of 5-phosphatase into the canalicular domain from the *trans* component of the Golgi apparatus. At present it is not possible to distinguish between these two options.

In liver, it is likely that receptor-activated hydrolysis of $\text{PtdIns}(4,5)\text{P}_2$ occurs at the sinusoidal plasma membrane, where the cell is bathed by blood-borne hormones such as adrenaline, vasopressin and angiotensin. Thus $\text{Ins}(1,4,5)\text{P}_3$ is generated at the sinusoidal pole of the cell, where the $\text{Ins}(1,4,5)\text{P}_3$ -releasable Ca^{2+} pool may be located (see Putney, 1986; Irvine *et al.*, 1988). $\text{Ins}(1,4,5)\text{P}_3$ is phosphorylated to $\text{Ins}(1,3,4,5)\text{P}_4$ by a cytosolic kinase (Kirk *et al.*, 1987). The majority of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ 5-phosphatase activities are membrane-bound (Storey *et al.*, 1984; Seyfred *et al.*, 1984; Joseph & Williams, 1985; Shears *et al.*, 1987b). Although the endosomal compartment appears to transport 5-phosphatase activity through the cell, the enzyme is inactive while in transit, owing to its intravesicular location (Fig. 1). The major site of 5-phosphatase activity is at the canalicular pole of the cell (Fig. 1). Thus the components of the inositol lipid signalling system are asymmetrically distributed through the hepatocyte, and this may establish concentration gradients across the cell, not only of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$, but also of their dephosphorylated products [$\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$ respectively]. The formation of any concentration gradient

will be opposed by diffusion of the various inositol phosphates, and binding sites for these compounds (Willcocks *et al.*, 1987) also influence their intracellular distribution. At present we do not have the techniques to measure the extent of intracellular concentration gradients of inositol phosphates.

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