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MONOACYLGLYCEROL ACYLTRANSFERASE ACTIVITY IN THE RAT LIVER PLASMALEMMA FRACTIONS

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Summary

Bile canalicular membranes and plasma membranes free of bile canalicular membranes were prepared from rat livers and their lipolytic activities were measured. Both preparations catalyzed hydrolysis and transacylation when monoacylglycerol and phosphatidylethanolamine were used as substrates. The specific enzymatic activity in the plasmalemma free of bile canalicular membranes was slightly higher than that in bile canalicular membranes. Neither preparation attacked the triacylglycerol of chylomicra, which indicates the lack of a lipoprotein lipase. Heparin and $CaCl_2$ stimulated the activities in both preparations. On the basis of these data, we suggest that monoacylglycerol acyltransferase can serve two distinct roles in the liver cell, depending upon the membrane fraction of association.

We have reported that plasma membranes prepared from rat liver contain a phospholipase A_1 [1]. Subsequent studies showed that this enzyme is released from the membrane by heparin [2] and that 1-[^{14}C]-oleoylglycerol (monoacylglycerol) is the preferred substrate [3]. This enzyme has been purified from post-heparin plasma [4] and shown to catalyze a transacylation reaction in which diacylglycerol is the product. Owing to the substrate specificity of the enzyme, it has been renamed monoacylglycerol acyltransferase [5]. In more recent studies, we found that monoacylglycerol acyltransferase can hydrolyze lipoprotein-associated monoacylglycerol and 1-[^{3}H]palmitoyl-2-acyl glycerophosphorylethanol-

Abbreviations: monoacylglycerol, 1-[^{14}C]oleoylglycerol; phosphatidylethanolamine, 1-[^{3}H]-palmitoyl-2-acyl glycerophosphoethanolamine; plasmalemma, plasmalemma free of bile canalicular membranes.

amine (phosphatidylethanolamine) radiolabeled in vivo (El Maghrabi, R., Waite, M., Sisson, P. and Rudel, L.L., unpublished observations). We therefore suggested that this enzyme is involved in the metabolism of remnant lipoproteins by the liver. This postulate was based on the assumption that the monoacylglycerol acyltransferase is localized in the plasmalemma free of bile canalicular membranes (plasmalemma) of the hepatocyte. The purpose of the study reported here was to test this assumption.

Yousef et al. [7,8] have isolated two subfractions of plasma membranes from rat livers, one rich in bile canalicular membranes and the other which comprised the remainder of the plasmalemma free of bile canalicular membranes. The preparations differed in their metabolic turnover, enzyme content and phospholipid composition. Therefore, these well characterized membrane preparations appeared to be well suited to test our postulate.

The plasma membrane fractions were prepared from male Wistar rats (Canadian Breeding Laboratories, Montreal, Canada), body weight approximately 150 g [7,8], in the laboratory of Dr. Yousef in Toronto and were shipped frozen to Winston-Salem where the assays were performed. One of three buffers (pH 7.4) was used during the isolation procedures: 1 mM $NaHCO_3$, 1 mM $NaHCO_3$ + 0.5 mM $CaCl_2$, or 1 mM phosphate (K_2HPO_4 - KH_2PO_4) buffer. Unless otherwise stated, 50 μg of plasma membrane protein were incubated with substrate for 10 min, as described before [3]. Lipoproteins radiolabeled in vivo with 2-[^{3}H]glyceroltrioleate were prepared and assayed as described elsewhere (El Maghrabi, R., Waite, M., Sisson, P. and Rudel, L.L., unpublished observations). The extracted products were separated by thin-layer chromatography using the ether/ligroin (b.p. 63—75° C)/formic acid system (35:65:1.5, by vol.).

The two preparations were first assayed using liposomes comprised of a mixture of phosphatidylethanolamine and monoacylglycerol [3]. Fig. 1 demonstrates that both preparations have hydrolytic and transacylation activity. The plasmalemma fraction, however, is about 50% more active than the bile canalicular membrane fraction, although in a few preparations made with bicarbonate the activity was distributed approximately equally between the two. Both preparations are 2—4 times more active on monoacylglycerol than on phosphatidylethanolamine. A similar finding was made using whole plasma membrane preparations [3]. The averages of results obtained from assays of 6 preparations (each obtained from 3 rats) are shown in Table I. Although no statistical analyses were done, owing to differences in the age of preparations, it is clear that both preparations have appreciable monoacylglycerol acyltransferase activity. No significant differences were found using the membranes isolated in the presence of $CaCl_2$ of phosphate buffer. By comparison, the specific activity of the 5′-nucleotidase in the bile canalicular membrane preparations was approximately three times greater than that of the plasmalemma [7]. Although clear-cut distinctions between the two preparations cannot be made, these data suggest that monoacylglycerol acyltransferase serves a primary role in the plasmalemma whereas 5′-nucleotidase would play a greater role in the bile canalicular membranes.

In part, the lower activity found with the bile canalicular membranes

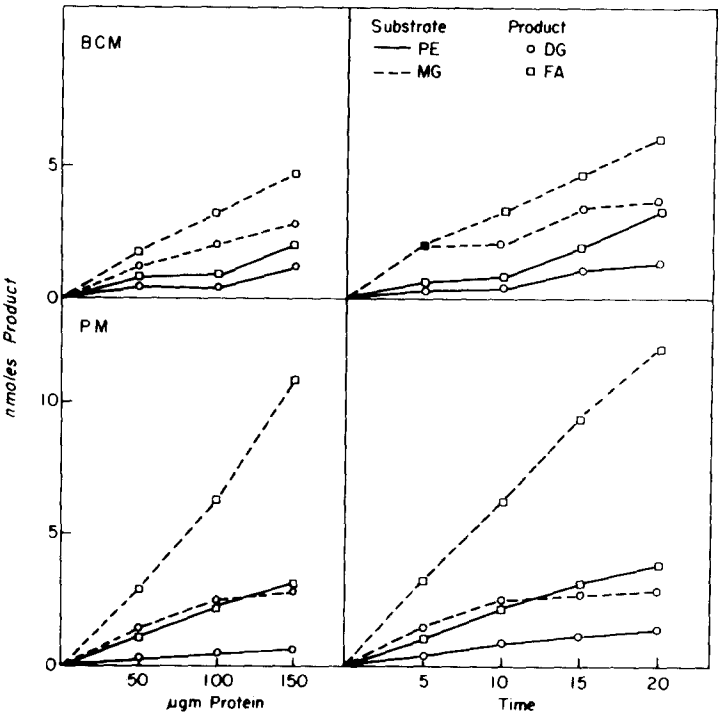

Fig. 1. Monoacylglycerol acyltransferase activity assayed as a function of time and amount of protein. The assay conditions are described in the text. BCM, bile canalicular membranes; PM, plasmalemma; PE, phosphatidylethanolamine; MG, monoacylglycerol; DG, diacylglycerol; FA, free fatty acid.

TABLE I

THE AVERAGE OF THE RESULTS OBTAINED USING SIX PREPARATIONS OF PLASMA MEMBRANE FRACTIONS

The conditions are described in the text.

Substrate	Membrane fraction		
	nmol total products		Ratio
	Bile canalicular membranes	Plasmalemma	Bile canalicular membranes/plasmalemma
PE	3.9	5.0	0.78
MG	8.6	12.8	0.67
Ratio (PE/MG)	0.45	0.39	

could be the result of substrate dilution by endogenous lipid as the bile canalicular membranes were shown to have twice the lipid to protein ratio of the plasmalemma [7]. It can be calculated from those data (Fig. 2) [7] that we added about 30 μg lipid in the bile canalicular membranes preparation and 15 μg lipid in the plasmalemma preparation. Since we used a total of 150 nmol of radiolabeled substrate, theoretically we could expect a 10% difference in activity based on the substrate dilution. Although

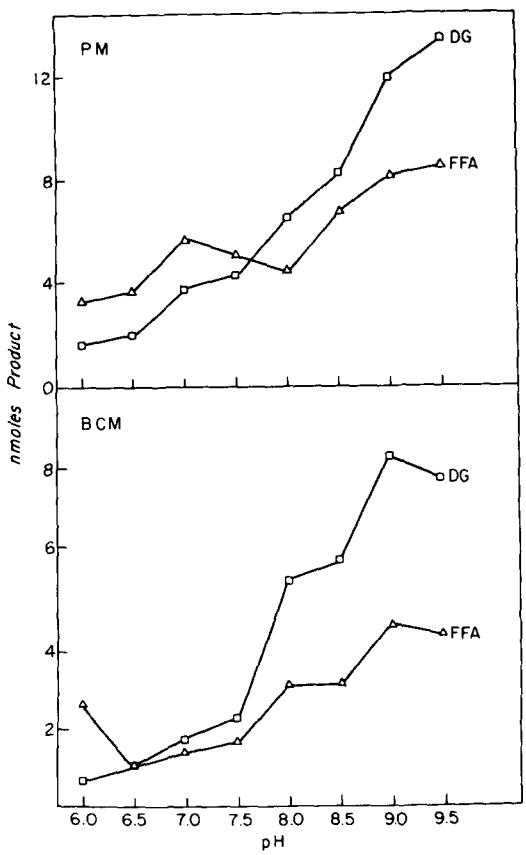

Fig. 2. Monoacylglycerol acyltransferase activity assayed as a function of pH. The assay conditions are described in the text except for the variation in pH; either Tris•HCl or Tris-maleate was used at the indicated pH values. PM, plasmalemma; BCM, bile canalicular membranes.

this dilution factor should include the contribution of each lipid, we conclude that our results are not appreciably affected by the differences in the lipid composition of the two preparations.

We also examined a number of known characteristics of the enzyme to determine if any distinctions between the two sources could be made. Fig. 2 shows the pH optima of the utilization of monoacylglycerol. The only significant difference that can be seen is between pH 6.0 and 8.0, well below the pH range of optimal activity. The finding that free fatty acid is the major product of the plasma from preparation activity below pH values of 8.0 could indicate the presence of a second lipolytic enzyme, either in the membrane itself or in a contaminating membrane fraction. Phosphatidylethanolamine was degraded in a similar pattern (results not shown).

Table II shows the effect of Ca^{2+} and heparin on monoacylglycerol acyltransferase activity. Ca^{2+} stimulated both preparations but was not essential, similar to our earlier observations [2]. Most surprisingly, heparin stimulated the enzyme in the bile canalicular membrane fraction, similar to its effect on the plasmalemma preparation. The significance of this is not

TABLE II

THE ASSAY CONDITIONS ARE DESCRIBED IN THE TEXT EXCEPT FOR THE INDICATED ADDITIONS OR DELETION

Membrane	Addition	nmol product from		
		Phosphatidylethanolamine	Monoacylglycerol	Lipoproteins neutral glyceride*
Plasmalemma	—	3.3	6.4	0.9
	$CaCl_2$ (5.0 mM)	5.8	6.8	0.9
	$CaCl_2$ (5.0 mM) plus heparin (50 μg)	7.8	15.5	0
Bile canalicular membranes	—	2.5	4.9	1.2
	$CaCl_2$ (5.0 mM)	4.0	6.2	1.0
	$CaCl_2$ (5.0 mM) plus heparin (50 μg)	5.7	11.4	0.6

*The degradation of the glycerol-labeled neutral lipid was measured by the release of radiolabeled glycerol.

yet understood. Also of interest is the finding that neither preparation had any significant lipoprotein lipase activity. The low amount of glycerol produced from triacylglycerol in chylomicra probably arises from the small amount of monoacylglycerol present (2—3%).

The plasmalemma as well as the bile canalicular membranes have monoacylglycerol acyltransferase, an enzyme postulated to be involved in remnant lipoprotein clearance (ref. 6 and El Maghrabi, R., Waite, M., Sisson, P. and Rudel, L.L., unpublished observations). If this were to be its sole function, we would expect it to be localized exclusively in the plasmalemma and any activity found in the bile canalicular membranes would be contamination. That monoacylglycerol acyltransferase is in the plasmalemma does indicate that it can play a role in remnant lipoprotein clearance by the liver. The monoacylglycerol acyltransferase found in the bile canalicular membranes cannot be ascribed to contamination, however. It appears, therefore, as if monoacylglycerol acyltransferase serves more than one function. There are at least two explanations that can be offered to account for its presence in the bile canalicular membranes; first it could serve a function in the secretion of phosphoglycerides into the bile duct [8] or second (and possibly related to the first), it could function in the Lands' cycle [9] to alter the molecular composition of the membranous phosphoglyceride. If the rapid secretion of the phospholipids from the bile canalicular membranes [7] involves metabolic interconversions at the membrane, monoacylglycerol acyltransferase could function in this process. The recent observation that acyl-CoA: 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase is in bile canalicular membranes [10] demonstrates that the Lands' cycle can function in bile canalicular membranes. Further studies on the influence of bile salts on monoacylglycerol acyltransferase are planned to test this hypothesis.

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References

1 Newkirk, J.D. and Waite, M. (1973) Biochim. Biophys. Acta 298, 562—576
2 Waite, M. and Sisson, P. (1973) J. Biol. Chem. 248, 7201—7206
3 Waite, M. and Sisson, P. (1973) J. Biol. Chem. 248, 7985—7992
4 Zieve, F.J., Freude, K.A. and Zieve, L. (1973) Fed. Proc. 32, 561
5 Waite, M. and Sisson, P. (1975) in Lipids (Paoletti, R., Porcellati, G. and Jacini, G., eds.), pp. 127—139, Raven Press, New York
6 Waite, M. and Sisson, P. (1976) Biochim. Biophys. Acta 450, 301—310
7 Yousef, I.M., Bloxam, D.L., Phillips, M.J. and Fisher, M.M. (1975) Can. J. Biochem. 53, 989—997
8 Yousef, I.M. and Fisher, M.M. (1976) Can. J. Biochem. 54, 1040—1046
9 Lands, W.E.M. and Crawford, C.G. (1976) in Enzymes of Biological Membranes (Martonosi, A., ed.), Vol. 2, pp. 1—85, Plenum Press, New York
10 Yousef, I.M., Fisher, M.M., Pierkarski, J. and Holub, B.J., (1977) Lipids, in the press