THE HYDROLYTIC AND TRANSACYLATION ACTIVITY OF THE PHOSPHOLIPASE A_1 PURIFIED FROM HUMAN POST-HEPARIN PLASMA

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Summary: This study demonstrated that the phospholipase A_1 purified from human post-heparin plasma catalyzes the same reactions (hydrolysis and transacylation) and utilizes the same substrates as the phospholipase A_1 obtained by heparin treatment of the plasmalemma of rat liver (Waite, M. and Sisson, P. (1973) J. Biol. Chem. 248, 7985). 1-acylglycerol was the preferred acyl donor and transacylation was the predominate reaction. The results strongly support our earlier conclusions that the phospholipase in plasma originates from the liver and that this enzyme is capable of using a variety of acyl acceptors, including water.

Introduction

In 1965 Vogel et al. reported the presence of a phospholipase A_1 (EC 3.1.1.4) in human post-heparin plasma which utilized the one-acyl moiety of egg yolk diacyl GPE (1). They found that this enzyme was capable of using alcohols as well as water as the acyl acceptor which produced the acyl ester of the acceptor alcohol. Zieve and Zieve recently provided evidence which suggested that the liver is the origin of this enzyme which is released into circulation upon the injection of heparin (2). The enzyme has been purified to homogeneity (3). It was demonstrated that the purified enzyme hydrolyzes 1-acylglycerol more rapidly than diacyl GPE (4).

The plasmalemma of rat liver contains a phospholipase A_1 that hydrolyzes diacyl GPE (5,6) and is displaced from purified rat liver plasmalemma by heparin (7). This enzyme utilizes the 1-acyl moiety of 1-acylglycerol, 1,2-

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Abbreviation: GPE, sn-glycero-3-phosphorylethanolamine

and 1,3 diacylglycerol, and 1-acyl GPE in addition to diacyl GPE (8). This preparation catalyzes a transacylation in addition to the hydrolytic reaction. Acyl acceptors with either primary or secondary hydroxyls can be utilized, providing the compounds are not too bulky (9). It was tentatively concluded that a single enzyme was responsible for these activities (8). This conclusion requires verification however, owing to the possible inferring activity of a triglyceride lipase (10).

The evidence provided by this study demonstrates that a single enzyme is capable of catalyzing both transacylations and hydrolysis and that the liver is source of the phospholipase found in the post-heparin plasma.

Methods

Post-heparin phospholipase A_1 was purified from human plasma by a method described in detail elsewhere (3). Briefly, the purification procedure involves chromatography of post-heparin plasma on QAE-Sephadex at pH 9.5, precipitation with ammonium sulfate, chromatography on DEAE-cellulose at pH 8.6, and centrifugation in sucrose gradients. The purified enzyme showed a single band on acrylamide gel electrophoresis in the presence and absence of sodium dodecyl sulfate. The enzyme used in the present studies hydrolyzed diacyl GPE with an initial specific activity of 4.6 μmoles$\cdot$min$^{-1}\cdot$mg^{-1} when assayed at a substrate concentration of 2×10^{-3} M in the presence of 0.5% Triton X-100. Enzymatic activity was measured using 50 μM of each lipid added as an ultrasonic suspension. Unless otherwise indicated, each reaction mixture contained 5.0 mM $CaCl_2$, 100 mM Tris-HCl, pH 9.0, and 2.5 μg of enzyme in 1.0 ml. The incubation temperature was 37^{o} and the usual incubation time was 10 min.

The reaction products were separated by thin layer chromatography in the ether-heptane-formic acid system (35:65:1.5 by volume) which separates triacylglycerol, fatty acid, 1,3-diacylglycerol, 1,2-diacylglycerol, monoacylglycerol, and phospholipid (both at or near the origin), or in the chloroform-ligroin (b.p. 63-75^{o})-acetic acid (70:30:4 by volume) followed by chloroform-methanol-water (70:30:4 by volume) which separates the neutral lipid, diacyl GPE and monoacyl GPE.

The labeled phospholipids were prepared as described earlier (7). The 1-[1-^{14}C]-oleoylglycerol was purchased from Applied Science Laboratories, Ann Arbor, Mich. The nonradioactive lipids were purchased from either Serdary Research Laboratories, Inc., London, Ontario, or from Nu-Chek Prep., Elysian, Minn.

Results and Discussion

Both diacyl GPE and 1-acylglycerol were hydrolyzed by the enzyme; the primary product when 1-acylglycerol was the substrate, however, was diacylglycerol (Figs. 1 and 2). Diacylglycerol was also produced from diacyl

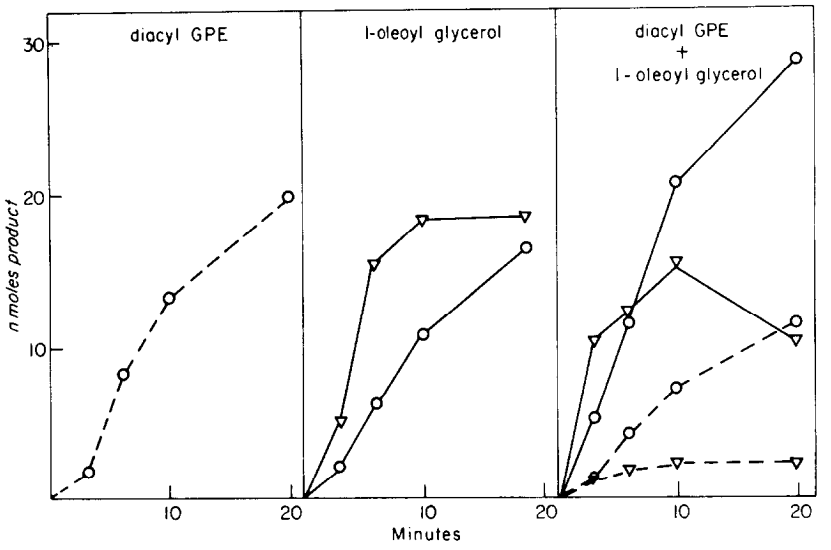

Figure 1. Phospholipase A_1 activity assayed as a function of time. Each mixture contained 2.5 μg of enzyme protein and was incubated for the indicated time intervals. The other conditions and procedures are described in Methods. The symbols are for fatty acid (O) and diacylglycerol (□). The solid line designates the products from 1-oleoylglycerol and the broken line designates the products from diacyl GPE.

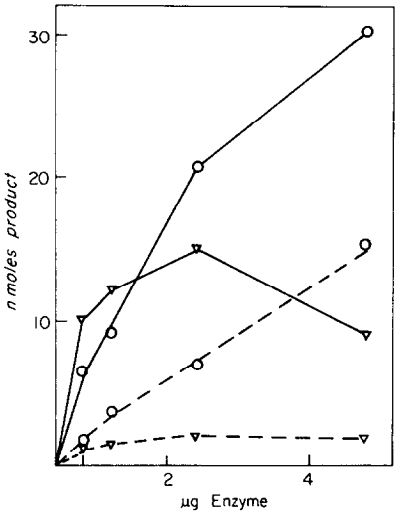

Figure 2. Phospholipase A_1 activity assayed as a function of the amount of protein. Each reaction mixture which contained both 1-[^{14}C] oleoylglycerol and 1-[^{3}H] diacyl GPE was incubated for 10 min. The other conditions and procedures are described in Methods. The symbols are for fatty acid (O) and diacylglycerol (□). The solid line designates the products from 1-oleoylglycerol and the broken line designates the products from diacyl GPE.

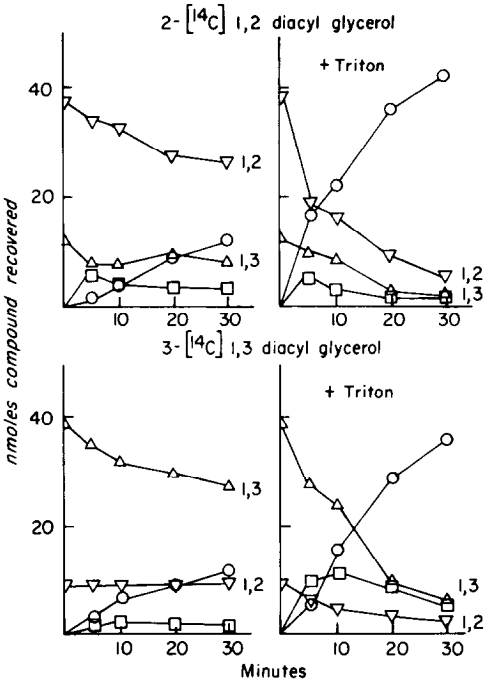

Figure 3. The hydrolysis of 1,2- and 1,3 diacylglycerol as a function of time. Each reaction mixture contained 2.5 μg of enzyme protein and was incubated for the indicated time intervals. The final concentration of Triton X-100 was 0.05% (by volume) in the tubes designated. The other conditions and procedures are described in Methods. The symbols are for fatty acid (O), monoacylglycerol (□), 1,2-diacylglycerol (▽), and 1,3-diacylglycerol (△).

GPE in the presence of the acyl acceptor, 1-acylglycerol. The presence of 1-acylglycerol depressed the utilization of diacyl GPE. With the concentrations of diacyl GPE and 1-acylglycerol employed in this experiment, the utilization of 1-acylglycerol was slightly stimulated. At 3 min the production of both diacylglycerol and fatty acid was increased whereas at the longer incubation periods the diacylglycerol produced was hydrolyzed which decreased the diacylglycerol and increased fatty acid recovered. Although not shown, this stimulatory effect was not noted when the ratio of diacyl GPE to 1-acylglycerol exceeded one. With a ratio of diacyl GPE to 1-acylglycerol of 2:1 inhibition of the utilization of 1-acylglycerol was observed, similar to the earlier observations (8). The activity of the 2.5 μg of the purified enzyme is comparable to that found with 44 μg of the soluble fraction from the rat liver

Substrate	Addition	Product (nmoles) Diacyl glycerol	Fatty acid	Diacyl propanediol	Diacyl glycol	Cetyl ester	Cholesterol ester	1,3-acyl, alkyl glycerol*	1,2-acyl, alkyl glycerol*
1-[^{3}H] palmitoyl-2-acyl GPE	1-palmitoyl-1,2-propanediol		19.2	2.3					
	1-palmitoyl-1,3-propanediol		22.5	2.3					
	1-palmitoyl glycol		24.4		2.2				
	cetyl alcohol		10.0			8.9			
	cholesterol		23.2				0.3		
	1-hexadecyl glycerol		7.7					7.7	1.0
	2-hexadecyl glycerol		6.7						10.6
	none	0	14.0						
1-[^{14}C] oleoyl glycerol	1-palmitoyl-1,2-propanediol	16.3	15.8	5.6					
	1-palmitoyl-1,3-propanediol	18.4	14.8	4.8					
	1-palmitoyl glycol	16.4	16.6		7.8				
	cetyl alcohol	14.6	15.8			7.0			
	cholesterol	20.0	20.3				0.8		
	1-hexadecyl glycerol	(11.9)	8.2					18.5	**
	2-hexadecyl glycerol	(29.0)	10.4					0	**
	none	20.0	21.0						

*The position of the ester and ether bond on the glycerol is determined by the position of the ether bond on the acyl acceptor added.
**The diacyl glycerols and 1,2-acyl, alkyl glycerols did not seperate on the chromatograms.

Table 1. The use of various acyl acceptors by the phospholipase A_1. All lipids were added as ultrasonic suspension prepared in water at a concentration of 50 μM. The amount of enzyme protein was 2.5 μg and the time of incubation was 10 min. All other conditions and procedures are described in Methods.

plasmalemma (Fig. 1, ref. 8). Substantially greater hydrolytic activities are observed when the enzyme is assayed at higher substrate concentrations in the presence of 0.5% Triton X-100 (3).

The purified enzyme was also capable of hydrolyzing both 1,2 diacylglycerol and 1,3 diacylglycerol (Fig. 3), as found with the soluble fraction from liver plasmalemma (8). Triton X-100 (0.05%) stimulated hydrolysis of diacylglycerol. Although 2-[^{14}C]-1,2 diacylglycerol was the substrate, fatty acid was the major radioactive product. This is due to migration of the [^{14}C] acyl group from position two to position one where it is susceptible to enzymatic hydrolysis (8). No triacylglycerol was produced from 1,2 diacylglycerol even though position three was available for acylation. When 3-[^{14}C] 1,3-diacylglycerol was the substrate, the main product was fatty acid. This was expected since the other product, monoacylglycerol, is rapidly hydrolyzed. When the reaction mixture which contained Triton X-100 was incubated 5 min, about twice as much acylglycerol as fatty acid was recovered. This could be the reflection of a preference of the enzyme for polyunsaturated acids since the [^{14}C] fatty acid is linoleic whereas the nonradioactive fatty acids in position one of diacyl GPC (from which the diacylglycerol was prepared) were predominately saturated.

We added to the assay mixtures several compounds which had been shown to be acyl acceptors for the phospholipase from liver plasmalemma (9). The enzyme used as acyl acceptor compounds with either primary or secondary hydroxyl groups (Table 1). The relative utilization of these compounds as acyl acceptors was similar with either 1-[^{14}C] oleoylglycerol or 1-[^{3}H] palmitoyl-2-acyl GPE as the acyl donor. These results substantiate our earlier finding that either position one or two can accept the acyl group from the acyl donor. Further, our conclusion that the enzyme cannot utilize as an acyl acceptor compounds which are relatively bulky (i.e., diacylglycerol or cholesterol) is reconfirmed.

The data presented in this communication provided strong support for these following conclusions: First, the phospholipase A_1 purified from post-heparin plasma is the same as that obtained by treatment of the plasmalemma

of liver. Second, the liver is the source of the post-heparin phospholipase. Third, the purified phospholipase can catalyze both hydrolytic and transacylation reactions as described earlier (8,9).

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References

1. Vogel, W. C., Ryan, W. G., Koppel, J. L., and Olwin, J. H. (1965) J. Lipid Res. 6, 335-340.
2. Zieve, F. J., and Zieve, L. (1972) Biochem. Biophys. Res. Commun. 47, 1480-1485.
3. Zieve, F. J., Freude, K. A., and Zieve, L., in preparation.
4. Zieve, F. J., Freude, K. A., and Zieve, L. (1973) Fed. Proc. 32, 561.
5. Newkirk, J. D., and Waite, M. (1971) Biochim. Biophys. Acta 225, 224-233.
6. Newkirk, J. D., and Waite, M. (1973) Biochim. Biophys. Acta 298, 562-576.
7. Waite, M., and Sisson, P. (1973) J. Biol. Chem. 248, 7201-7206.
8. Waite, M., and Sisson, P. (1973) J. Biol. Chem. 248, 7985-7992.
9. Waite, M., and Sisson, P. (1974) J. Biol. Chem. 249, 6401-6405.
10. Assmann, G., Krauss, R. M., Fredrickson, D. S., and Levy, R. I. (1973) J. Biol. Chem. 248, 1992-1999.