ISOENZYMES OF AN ACYLTRANSFERASE FROM RABBIT MAMMARY GLAND: SOLUBILIZATION OF THE MICELLE-SPECIFIC SPECIES WITH TRITON X-100

Martin Caffrey* and John E. Kinsella

College of Agriculture and Life Sciences, Stocking Hall, Cornell University

Ithaca, New York 14853

Received May 26,1976

SUMMARY

The micelle-specific palmityl-CoA:monopalmityl-sn-glycerol 3-phosphate palmityltransferase isoenzyme from lactating rabbit mammary gland microsomes is selectively solubilized in Triton X-100 but not Tween 80. Both detergents inactivate the monomer-specific isoenzyme. The possibility that, in vivo, physiological surfactants regulate the relative activities of these two isoenzymes is discussed.

The enzyme palmityl-CoA:monopalmityl-sn-glycerol 3-phosphate palmityltransferase [lysophosphatidic acid acyltransferase or LPAT (EC 2.3.1.-)] catalyses the acylation of monopalmityl-sn-glycerol 3-phosphate (lysophosphatidic acid or LPA) to dipalmityl-sn-glycerol 3-phosphate (phosphatidic acid) with palmityl-CoA as donor. Under standardized assay conditions (pH 7.4) both substrates are anionic amphiphiles and within the acceptor concentration range used to define the LPA saturation curve (V/S_a curve) of this enzyme, a monomer-micelle transition occurs [1]. The LPAT from rabbit mammary gland microsomes is apparently composed of two isoenzymic species, denoted α and β, respectively [1]. The α form is active with monomeric or dispersed substrates molecules and inhibited by micelles whereas the β isoenzyme is active with micelles but not monomers. Thus, the V/S_a profile of these two isoenzymes corresponds, respectively, to Type III and IV saturation kinetics as defined by Gatt et al. [2]. In microsomes from lactating mammary tissue, both species are active and when the Vmax-β/

*To whom correspondence should be addressed

Vmax-α ratio is greater than 0.5, the V/S_a curve becomes biphasic at the critical micelle concentration (cmc) of the substrate [3].

In a continuing study aimed at confirming the existence of two LPAT isoenzymes in rabbit mammary microsomes and examining their properties further, we have solubilized the micelle-specific (LPAT-β) species from the particulate fraction of this tissue. As a result of the differential response of the LPAT isoenzymes to aggregated amphipaths the possibility that, in vivo, physiological surfactants regulate the relative activities of these two isoenzymes is briefly discussed.

MATERIALS AND METHODS

The following procedures were carried out on ice or at the temperature indicated. Finely chopped mammary tissue from a rabbit (Dutch Belted variety) 9 days post-partum was homogenized for 60-s at high speed in 8 vols. of 0.25M sucrose on a two-speed Waring Blendor drive unit with stainless steel semimicro Eberbach homogenizing jar. Tissue that had associated on homogenization was ground in a mortar with glass beads (0.45-0.5 mm diameter) and returned to the homogenate for an additional 60-s homogenization. This crude homogenate was then centrifuged at 15,000g (r_{av} 4.25") for 20 min at 4°C in a Sorval Superspeed RC2-B centrifuge with Sorval type SS-34 rotor. The supernatant was carefully decanted through four layers of cheesecloth and centrifuged at 44,000g (r_{av} 9 CM) for 1-h at 4°C in a Beckman L2-65 ultracentrifuge with Beckman Type 21 rotor. The microsomal fraction was collected after careful decantation of the post-microsomal supernatant and suspended in 0.25M sucrose to 20-30 mg protein/ml using a Ten Broeck tissue homogenizer. After sonication for 1 min in a Model 8845-3 Sonicator (Cole-Palmer Ultrasonic Cleaner) the microsomal suspension was analyzed for protein using a modification of the Lowry method [1] and assayed for transacylase activity spectrophotometrically [1].

Solubilization of the LPAT-β isoenzyme was achieved in a solution containing microsomes (8 mg protein/ml) and Triton X-100 (4 mg/ml). The solution was stored on ice for 30 min and centrifuged at 100,000g (r_{av} 5.95 CM) for 2-h at 4°C in a Beckman L2-65 ultracentrifuge with Beckman Type 40 rotor. The supernatant was carefully drawn off using a pasteur pipette and protein and enzyme activity in this fraction was measured as above.

RESULTS AND DISCUSSION

At the outset, it is emphasized that solubilization is used in the present context to indicate enzymatic activity not sedimented by centrifugation in a fixed-angle rotor (Beckman, Type 40) for 2-h at 100,000g (r_{av} 5.95 CM) and 4°C in a solution containing specified levels of added detergent. Further, particles of less than $60S°_{20,w}$ will remain in the supernatant of an aqueous suspension upon centrifugation at 20°C under the solubilization conditions cited under "Materials and Methods."

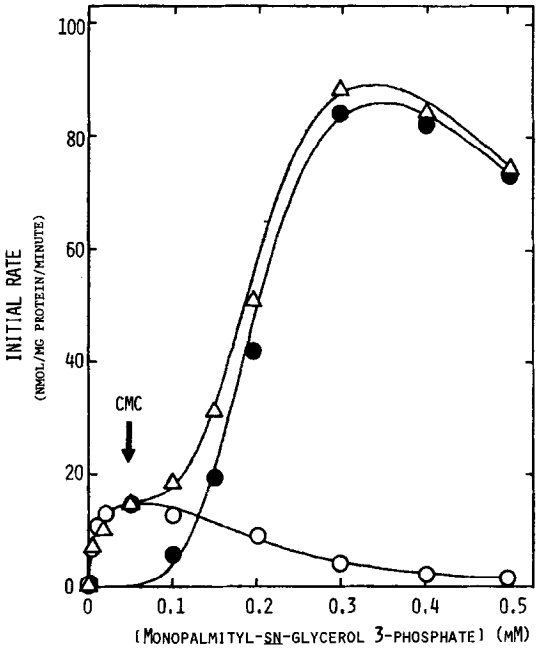

Figure 1. Substrate saturation curves obtained with rabbit mammary microsomes containing both LPAT-α and β isoenzymes (△—△, curve A) and LPAT-α alone (○—○, curve B). Curve C (●—●) represents the graphically resolved saturation curve for LPAT-β alone. All curves were generated by computer according to rate equations presented previously [1]. Reaction mixtures contained Tris-HCl buffer, 70 mM, pH 7.4; 4,4'-dithiodipyridine (Aldrich), 0.3 mM; palmityl-CoA (P-L Biochemicals), 18 μM; microsomal protein, 0.23 mg/ml; and monopalmityl-sn-glycerol 3-phosphate (Serdary) as indicated. Specific activity is expressed as nmol palmitate transesterified/mg protein/min. The approximate critical micelle concentration (cmc) of the substrate is indicated. [Reproduced from M. Caffrey et al., FEBS Lett. 52, 116-120, 1975 by permission of the authors and of the publisher].

The V/S_a curve for untreated microsomes from lactating rabbit mammary gland containing both LPAT-α and β isoenzymes is presented in Fig. 1 (curve A). The curve becomes biphasic at the cmc of the substrate (≈50 μM LPA or 70 μM total surfactant, i.e., palmityl-CoA plus LPA) illustrating the differential response of the LPAT isoenzymes to monomeric and micellar substrates. The V/S_a curve for LPAT-α alone and that for LPAT-β obtained by graphical resolution [1] are included in Fig. 1 to facilitate discussion.

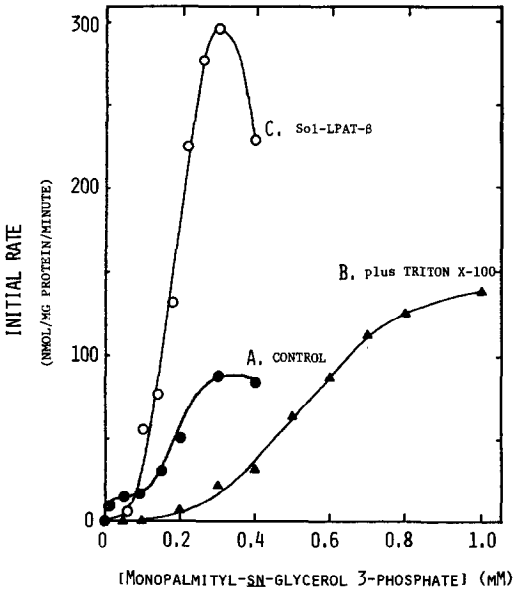

Figure 2. Dependence of solubilized and particulate palmityl-CoA:monopalmityl-sn-glycerol 3-phosphate palmityltransferase activity on monopalmityl-sn-glycerol 3-phosphate concentration. Control curve A was taken from Fig. 1 (curve A). In B the reaction mixture was as in A with Triton X-100, 4 mg/ml. Reaction mixture in C was as in A with solubilized microsomal protein, 0.044 mg/ml; and Triton X-100, 0.4 mg/ml. Membrane solubilization in Triton X-100 is described under "Materials and Methods." The control curve A was generated by computer as described previously [1].

Triton X-100 has been shown to effectively solubilize membranal proteins without loss of function [4,5,6] and was used to solubilize LPAT-β from the particulate fraction of rabbit mammary gland. Prior to carrying out the solubilization experiments, however, microsomes were initially assayed for transacylase activity in the presence of Triton X-100 to determine the immediate effect of this detergent on the LPAT isoenzymic pair. The results (Fig. 2, curves A and B) show that including Triton X-100 in the reaction medium brought about complete loss of LPAT-α activity and a significant enhancement in the specific activity of LPAT-β. This contrasting sensitivity of LPAT-α and β to Triton X-100 further supports the contention that LPAT in rabbit mammary microsomes is composed of two isoenzymes.

When mammary microsomes were subjected to the solubilization procedure outlined above, 40% of the microsomal protein was recovered in the high-speed supernatant. This fraction was rich in β activity, with a maximum specific activity 3-times that of the control (Fig. 2, curve C). Combining these results, it is apparent that solubilization and partial purification of the LPAT-β isoenzyme was achieved. Thus, the method described represents a simple and rapid procedure for the selective solubilization of LPAT-β from rabbit mammary microsomes.

The dependence of solubilized LPAT-β (Sol-LPAT-β) activity on LPA concentration is typically sigmoid (Fig. 2, curve B and C) as predicted in an earlier study [1].

Much evidence has been obtained which suggests that LPAT-β is specific for substrates in the micellar form [1,3]. Thus, the dependence of initial velocity on Sol-LPAT-β enzyme concentration (V/E) should be linear from the origin and level off above that concentration of added enzyme where nonspecific substrate binding reduces substrate concentration below the cmc [3,7]. This relationship was examined for Sol-LPAT-β at a constant level of Triton X-100 with enzyme concentration being the only variable. The recorded V/E curve (Fig. 3), however, intercepted the abscissa in the first quadrant, was parabolic to 0.44 mg protein/ml and linear up to 1.1 mg/ml. This result suggests that an inhibitory or toxic substance is present in the assay medium which combines reversibly with the enzyme to give an inactive complex [8]. Therefore, at low levels of added enzyme almost complete inhibition is observed but at higher concentrations the percentage of total enzyme inhibited becomes increasingly smaller giving rise to the observed V/E curve. Alternatively, a protein concentration-dependent association of protomeric subunits into active multimers may also explain these data. However, the exact mechanism by which this atypical behavior is effected must await further experimentation.

The response of the LPAT isoenzymes to Tween 80 was similar to that reported above for Triton X-100, i.e., LPAT-α activity was eliminated and that of the β

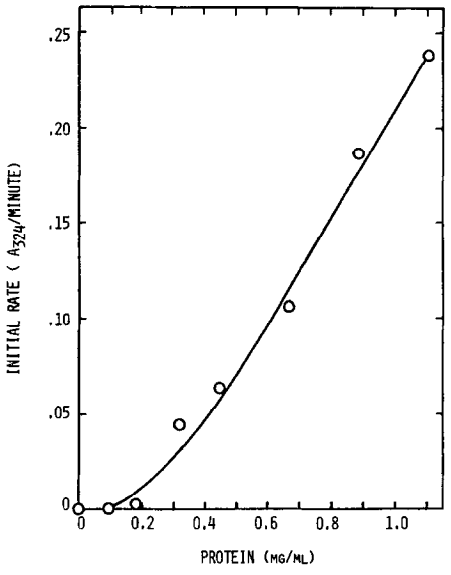

Figure 3. Dependence of palmityl-CoA:monopalmityl-sn-glycerol 3-phosphate palmityltransferase reaction rate on the concentration of solubilized microsomal protein. Reaction mixture as specified in Fig. 1A, with monopalmityl-sn-glycerol 3-phosphate, 0.18 mM; Triton X-100, 0.1 mg/ml; and protein as indicated.

isoenzyme significantly enhanced when microsomes were incubated with this detergent. However, although Tween 80 is an efficient solubilizer of particulate protein [6], in contrast to Triton X-100, it was ineffective in the solubilization of an active LPAT-β.

The available data indicates that LPAT-α is specific for monomeric substrates and inhibited by the micellar form. In addition, this isoenzyme is inactivated by Triton X-100 and Tween 80, both at concentrations above their respective cmc's, suggesting that LPAT-α is sensitive to all surfactant micelles regardless of chemical identity and composition. This does not appear to be the case for LPAT-β. It is postulated, therefore, that in the mammary cell, LPAT-α is inactivated upon accumulation and subsequent aggregation of endogenous surfactants such as diacylglycerides and phospholipids. However, as depicted in Fig. 4, the α-glycerolphosphate pathway of glycerolipid synthesis may continue to operate under these conditions since LPAT-β is active in the presence of amphiphilic

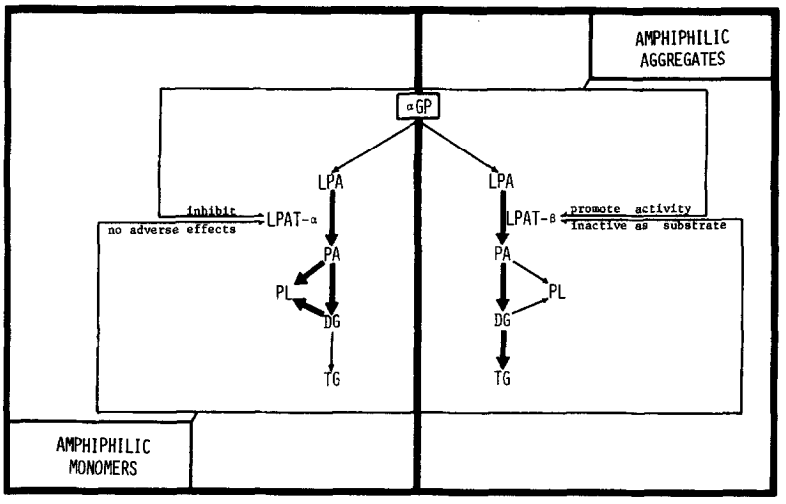

Figure 4. A tentative scheme showing the reciprocal control of the LPAT isoenzymes involved in glycerolipid synthesis. In the right and left hand sectors, respectively, amphiphiles are present at concentrations above and below their corresponding cmc's. In either case the favored pathway is emphasized by heavy arrows (see text for details). Abbreviations used are αGP, α-glycerolphosphate; LPA, lysophosphatidic acid; PA, phosphatidic acid; PL, phospholipid; DG, diacylglyceride; and TG, triacylglyceride.

aggregates. A reciprocal control of this pathway at the LPAT level is, therefore, envisaged whereby the degree of association of these amphiphiles modulates the relative activities of the α and β isoenzymes (Fig. 4). In order that this reciprocal control be of advantage to the cell, it is proposed that the two LPAT isoenzymes differ not only with respect to monomer-micelle specificity, but at some additional level(s) also, for example, subcellular compartmentation and/or substrate specificity. This is consistent with our previous suggestion that the two isoenzymes may be involved in the synthesis of phosphatidic acid for two separate diacylglyceride pools, one for phospholipid synthesis and a second, for the generation of secretory triacylglycerides [1]. In this connection, it is instructive to note that Ontko [9] has shown the synthesis of di- and triacylglycerides in isolated rat liver cells to increase in a sigmoid manner as phospholipid formation approaches saturation. These observations

are compatible with the above postulates (see Fig. 4) if LPAT-α, the monomer-specific isoenzyme, is operating in the direction of phospholipid synthesis and LPAT-β, the micelle-specific form, is restricted to triacylglyceride formation.

ACKNOWLEDGEMENTS

This work was supported by National Science Foundation Grant No. 37174.

REFERENCES

1. Caffrey, M., Infante, J. P., and Kinsella, J. E. (1975) FEBS Lett. 52, 116-120.
2. Gatt, S., Barenholz, Y., Borkovski-Kubiler, I., and Leibovitz-Ben Gershon, Z. (1972) Adv. Exp. Med. Biol. 19, 237-256.
3. Caffrey, M. (1976) Thesis, Cornell University, Ithaca, N. Y. 14853.
4. Razin, S. (1972) Biochim. Biophys. Acta 265, 241-296.
5. Shiu, R. P. C., and Friesen, H. G. (1974) J. Biol. Chem. 249, 7902-7911.
6. Helenius, A., and Simons, K. (1975) Biochim. Biophys. Acta 415, 29-79.
7. Barden, R. E., and Cleland, W. W. (1969) J. Biol. Chem. 244, 3677-3684.
8. Dixon, M., and Webb, E. C. (1964) Enzymes, p. 58, Academic Press, New York.
9. Ontko, J. A. (1972) J. Biol. Chem. 247, 1788-1800.