Short Communications

Acylation of Lysolecithin to Lecithin by a Brush-Border-Free Particulate Preparation from Rat Intestinal Mucosa

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For several years we have been investigating the intracellular localization and role of the mucosal enzymes hydrolysing and synthesizing cholesteryl and retinyl esters, and have demonstrated that all of the sterol ester hydrolase and most of the retinyl ester hydrolase of rat intestine can be recovered from the isolated brush border (David, Malathi & Ganguly, 1966; David & Ganguly, 1967). In an extension of this work we have found that considerable amounts of the phospholipase A activity of rat intestinal mucosa is also present in the brush border (P. V. Subbaiah & J. Ganguly, unpublished work).

Recent work by Nilsson & Borgström (1967) with slices and sacs of rat and hamster intestine and by Scow, Stein & Stein (1967) and by Nilsson (1968) with intact rats has shown that during absorption lecithin (phosphatidylcholine) is hydrolysed to lysolecithin (1-acylglycerophosphorylcholine), which in turn is reacylated in the intestinal mucosa before entering the lymph. However, attempts to demonstrate direct acylation of lysolecithin with cell-free preparations from rat intestinal mucosa have been unsuccessful (Webster, 1965), although this reaction has been shown with enzyme preparations from many other rat tissues (Lands, 1960; Webster, 1965). One reason for this failure could be the presence of a powerful phospholipase A activity in these mucosal preparations. Since we have been able to localize the phospholipase A activity in the brush border, it appeared that it might be possible to demonstrate the acylation of lysolecithin to lecithin with a brush-border-free mucosal preparation. Using such a preparation we have now shown the presence of a powerful acylglycerophosphorylcholine-acyl-CoA acyltransferase in rat intestinal mucosa.

Lysolecithin was prepared by the procedure of Long & Penny (1957) from egg-yolk lecithin, and [1^{14}C]oleyl-CoA was obtained enzymically by the method of Kornberg & Pricer (1953) as modified by Bloomfield & Bloch (1960). The brush border was isolated from rat intestine by following the procedure of Miller & Crane (1961). The supernatant left after the removal of the brush border was

centrifuged at 100 000g for 1 hr. in a Spinco model L ultracentrifuge to sediment the brush-border-free particles. The sediment was resuspended in 0.25 M-sucrose and the suspension was used as the source of the enzyme. The reaction mixtures and the conditions of incubations are described in Table 1. At the given time the reactions were stopped by the addition of 2 ml. of methanol, after which the lipids were extracted by the procedure of Bligh & Dyer (1959). The lecithin was separated on silica-gel plates (Subbaiah, Raghavan & Ganguly, 1968) with chloroform-methanol-water (65:25:4, by vol.) as the developing solvent and the spots were scraped out and eluted (Abramson & Blecher, 1964). Portions of the eluates were plated for counting of the radioactivity (Murthy, David & Ganguly, 1963). Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

As shown in Table 1, the brush-border-free particles actively incorporated [1^{14}C]oleic acid into lecithin. The incorporation was dependent on ATP and CoA, suggesting that activation of the fatty acid is necessary for its incorporation, and this was substantiated by the fact that [1^{14}C]oleyl-CoA was incorporated equally well in the absence of the cofactors and that the addition of ATP did not enhance the incorporation. There was poor incorporation in the absence of lysolecithin, which indicates a net synthesis of lecithin by the acylation of lysolecithin.

In such reactions one would expect the rate of incorporation of oleyl-CoA to be much faster than that of free oleic acid. But in these experiments they were similar, and this might have been due to the use of large amounts of enzyme and to the long periods of incubation. In subsequent experiments, where much smaller amounts of enzyme (0.1–0.3 mg. of protein) were used and the reactions were carried out for very short periods of time (5 min.), the initial rates of incorporation of free oleic acid and of oleyl-CoA were 120–300 and 3640 nmoles/hr./mg. of protein respectively. If it is assumed that the step of acylation is much faster than the activation step, then the rate of incorporation of free oleic acid, as found in our experiments, compares

Table 1. *Acylation of lysolecithin with oleic acid or oleyl-CoA by rat intestinal mucosa*

The complete system with oleic acid included: 0.1 μ mole of [$1-^{14}\text{C}$]oleic acid (215 c.p.m./nmole), 0.8 μ mole of lysolecithin, 10 μ moles of ATP, 30 μ moles of MgCl_2 , 5 μ moles of GSH, 50 μ moles of NaH_2PO_4 – Na_2HPO_4 buffer, pH 7.4, and 1.2 mg. of protein of brush-border-free particles, in a final volume of 0.8 ml. The complete system with oleyl-CoA consisted of 0.1 μ mole of [$1-^{14}\text{C}$]oleyl-CoA (195 c.p.m./nmole), 0.8 μ mole of lysolecithin, 30 μ moles of MgCl_2 , 50 μ moles of NaH_2PO_4 – Na_2HPO_4 buffer, pH 7.4, and 1.2 mg. of protein of brush-border-free particles in a final volume of 0.8 ml. Incubations were carried out for 1 hr. at 37° and subsequent extraction and analysis were as described in the text. The values given are averages of duplicate samples.

	Fatty acid incorporated (nmoles/hr./mg. of protein)
[$1-^{14}\text{C}$]Oleic acid	
Complete system	38.5
Complete system – ATP	0.78
Complete system – CoA	1.70
Complete system control (0 min. incubation)	0.93
[$1-^{14}\text{C}$]Oleyl-CoA	
Complete system	41.7
Complete system + 10 μ moles of ATP	45.5
Complete system – lysolecithin	6.45
Complete system control (0 min. incubation)	1.05

favourably with the activity of the acyl-CoA synthetase of the microsomal fraction from rat mucosa as reported by Senior & Isselbacher (1960).

In separate experiments the position the labelled fatty acid had occupied in the synthesized lecithin was determined. For this purpose several labelled lecithin samples, isolated after incubation, were pooled and subjected to the action of *Crotalus*

adamanteus venom (Long & Penny, 1957). The fatty acids liberated and those of the lysolecithin formed were analysed and almost all the radioactivity of the lecithin was accounted for in the fatty acids liberated, which established that the labelled oleic acid was incorporated into the β -position of lecithin.

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