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ACCESSIBILITY OF LYSOLECITHIN IN CATECHOLAMINE SECRETORY VESICLES TO ACYL COA:LYSOLECITHIN ACYL TRANSFERASE

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SUMMARY: Lysolecithin (monoacylglycerophosphorylcholine) accounts for 13 to 20% of the lipid phosphorous of the bovine adrenal catecholamine secretory vesicles (chromaffin granules). We have incubated purified vesicles with [1-^{14}C] oleyl coenzyme A and rat liver microsomes containing acyl coenzyme A: monoacylglycerophosphorylcholine acyl transferase to determine the accessibility of the granule membrane lysolecithin to another membrane. No acylation of lysolecithin occurs when the chromaffin granules are intact. The accessibility of the granule membrane lysolecithin increases markedly when the vesicles are broken.

The high content of lysolecithin in the membranes of bovine chromaffin granules (1,2) has prompted the suggestion that this phospholipid may play a role in the fusion of the chromaffin granule membrane with the plasma membrane of the cell during exocytosis (1). Lysolecithin has been shown to cause erythrocytes to fuse (3). For lysolecithin to be involved in exocytosis, it would be expected to be located on the outside surface of the granule membrane. As a probe to determine whether the lysolecithin is on the exterior surface of the chromaffin granule membrane, we have looked at how accessible the lysolecithin is to the membrane-bound enzyme, acyl coenzyme A:monoacylglycerophosphorylcholine acyl transferase of rat liver microsomes.

MATERIALS AND METHODS

Chemicals were obtained from the following sources: [1-^{14}C] oleic acid, New England Nuclear; coenzyme A, and Trizma Base, Sigma Co.; Ficoll, Pharmacia Co., deuterium oxide, 99.8 atom %, Bio-rad Lab.; sucrose and organic solvents, Fisher Chemical Co.; silica gel H, Applied Science Labs.

[1-^{14}C] Oleyl coenzyme A was prepared by the method of Okuyama et al. (4).

Protein was precipitated from samples with 8% trichloroacetic acid, and the pellet assayed for protein by the method of Lowry et al. (5).

Chromaffin granules were prepared by two methods which differ only in the final step of preparation (6). The method of Smith and Winkler (7) involves centrifugation of the 2.42 x 10^5 g. min pellet through hyperosmotic 1.6 M sucrose to obtain a purified chromaffin granule pellet (sucrose granules).

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Alternatively, the 2.42 x 10^5 g. min pellet may be centrifuged through a solution of 0.27 M sucrose, 1.9% (w/v) Ficoll in deuterium oxide (8) to obtain isoosmolar chromaffin granules (Ficoll granules). Both preparation yield granule fractions which contain low levels of contaminating marker enzymes (6). Broken granules were prepared by diluting sucrose granules, or Ficoll granules which had been made 1.6 M with respect to sucrose, 1:20 (v/v) in the reaction buffer. Granules so treated release 95% of their catecholamine to the medium, and show a marked decrease in density on sedimentation through sucrose solutions.

Rat liver microsomes were prepared by the method of Eibl et al. (9). To remove acyl-CoA hydrolase, the microsomes were treated with sodium deoxycholate and bovine serum albumin in the proportions described by Reitz et al. (10). This mixture was layered on 0.63 M sucrose and centrifuged for 1.0 x 10^7 g. minutes. The pellet was discarded and the supernatant was diluted to 0.25 M sucrose and centrifuged for 1.0 x 10^7 g. minutes. The supernatant was discarded and the pellet resuspended in 0.3 M sucrose, 0.1 M Tris pH 7.4. These washed rat liver microsomes were assayed spectrophotometrically for acyltransferase activity by the method of Lands & Hart (11) with optimal levels of lysolecithin as substrate. Washed microsomes were assayed with and without lysolecithin in order to calculate the percentage of hydrolase activity in the preparation. Usually 2 to 6% of the apparent acyltransferase activity as shown by the spectrophotometric assay was due to acyl coenzyme A hydrolase activity.

Incubations were carried out at 4° and contained 0.27 M sucrose; 0.09 M Tris·HCl pH 7.4; 25 µg of granule protein (containing approximately 4 nmol of lysolecithin), washed rat liver microsomes containing 4.5 to 6.5 milliunits of acyltransferase activity, 7.5 nmol of [1-^{14}C] oleyl coenzyme A (4 nCi per nmol in a final volume of 0.25 ml. Both sucrose granules and Ficoll granules have been used as broken granules. Ficoll granules were commonly used as intact granules, but sucrose granules could also be used if the reaction was carried out in 1.6 M sucrose. It was shown through spectrophotometric assays that 1.6 M sucrose did not inhibit acyltransferase activity. The reaction was initiated with [1-^{14}C] oleyl coenzyme A and stopped by the addition of 1.0 ml of chloroform:methanol, (2:1). The lower phase was removed and the water layer was washed twice with 1 ml of Folch lower phase (12). The organic layers were combined and the solvent evaporated. The residue was dissolved in a small volume of chloroform:methanol, (2:1) and applied to a thin layer plate coated with silica gel H, 250 µm thick. First the plate was developed with 15% methanol in diethyl ether to move free fatty acid to the upper part of the plate, then it was developed in the same direction with chloroform:methanol water, (65:30:5) to within 5 cm of the top, to separate the phospholipids. Lipids were visualized under ultraviolet light after spraying with 0.25% dichloroflouroscein in ethanol. The lecithin spot was scraped into a 7 ml scintillation vial and 3.5 ml of toluene based scintillant was added. Radioactivity was determined in a Beckman LS-100C scintillation counter.

RESULTS AND DISCUSSION

As shown in Figure 1 and Table 1, only a low level of acylation of lysolecithin occurs in intact chromaffin granules. A significant acylation of lysolecithin does occur with broken granules. Comparison of the results with intact sucrose granules and intact Ficoll granules shows that there is no significant difference in the acylation of the lysolecithin of intact granules due to different osmotic conditions. The highest level of acylation reported

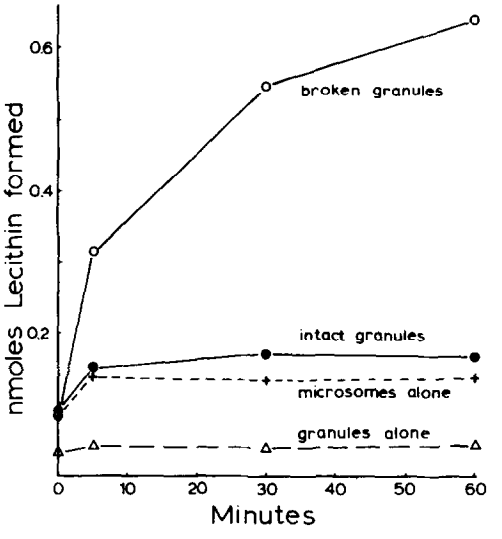

Figure 1. Chromaffin granules were incubated at 4° in 0.27 M sucrose, 0.09 M Tris·HCl pH 7.4, with 4.5 milliunits of acyltransferase activity (microsomes), and 7.5 nmol of [1-^{14}C] oleyl coenzyme A (4 nCi per nmol) in a final volume of 0.25 ml. 25 µg of granule protein were used. Broken granules, (o), intact granules, (o), broken granules with no microsomes, (Δ), and microsomes with no granules, (+), were incubated for 0, 5, 30 and 60 minutes.

Table 1

Experiment	Preparation of Chromaffin Granules		nmol of lecithin produced in 60 minutes at 4°C	
	Intact	Broken	Intact	Broken
1	Ficoll	Sucrose	0.02	0.52
2	Ficoll	Ficoll	0.08	0.52
3	Sucrose	Sucrose	0.04	1.3

Chromaffin granules were incubated at 4° in sucrose solutions containing 0.09 M Tris·HCl pH 7.4, with 4.5 milliunits of acyltransferase activity (microsomes), and 7.5 nmol of [1-^{14}C] oleyl coenzyme A (4 nCi per nmol) in a final volume of 0.25. 25 µg of granules protein were used.

For sucrose granules to be assayed as intact granules, the reaction was incubated in buffered 1.6 M sucrose. Intact Ficoll granules were assayed in buffered 0.27 M sucrose and all broken granules were prepared as described in Methods and assayed in buffered 0.27 M sucrose.

in Table 1 represents about one third of the original lysolecithin content of the granules.

The results show clearly that the accessibility of the membrane-bound lysolecithin to the acyl transferase enzyme is greatly increased when the granules are broken. There are several possible explanations for the inaccessibility of lysolecithin in the intact chromaffin granule. 1) It may be located on the inner surface. 2) The intact granule may present a surface conformation on which lysolecithin is not easily recognized. 3) The lysolecithin may be in a location, such as a cluster of charged groups, which prevents the approach of the acyl transferase. If the lysolecithin is on the inside surface of the membrane rather than the exterior surface, it seems unlikely that it is involved in the primary event in the fusion of the granule membrane with the plasma membrane. Since lysolecithin is 13 to 20% of the total membrane bound lipid phosphorous (13) this asymmetric distribution would make the phospholipid of the inner surface of the membrane about one third lysolecithin. Such a high content of lysolecithin should have some important role in the function of this membrane if it reflects the in vivo situation. Measurements of the hydrodynamic properties of intact granules by intensity fluctuation spectroscopy show that chromaffin granules prepared in 1.6 M sucrose are smaller and less polydisperse than those prepared in Ficoll (14). Table 1 shows that there is little difference in amount of acylation between intact sucrose granules and intact Ficoll granules. This suggests that shrinkage of the intact granules in 1.6 M sucrose or possible inclusion of immature granules in the Ficoll preparation is not a factor in determining the accessibility of lysolecithin for acylation. On the other hand, broken granules may have edges on which lysolecithin may accumulate in a conformation which makes it more available to the transferase. Electron micrographs of broken granules suggest that they form vesicles with holes in them, or sheets of membrane (6).

To our knowledge this is the first attempt to determine the orientation of lysolecithin in a membrane. This is also a potentially useful model for studying the interactions of membrane-bound enzyme with a membrane substrate.

Further experiments are in progress to discriminate among some of the

possible explanations for the inaccessibility of the lysolecithin in the intact granules.

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