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Use of trypsin to eliminate residual snake-venom phospholipase A activity in lysolecithin prepared from ovolecithin

MARPLES AND THOMPSON[1] have reported that lysolecithin prepared from ovolecithin by the action of cottonmouth mocassin venom contained residual phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) in an active state, confirming similar observations of SAUNDERS AND THOMAS[2]. These workers further reported that crystallization of lysolecithin from hot ethanol resulted in complete removal of the phospholipase activity as tested both by CO_2 evolution and by acid titration.

Under certain conditions, however, we have found that the residual phospholipase activity is still demonstrable after 5–6 recrystallizations from hot ethanol of lysolecithin prepared from ovolecithin treated with naja naja snake venom. When equal molar concentrations of the above prepared lysolecithin and ovolecithin were incubated at 37° in the presence or absence of buffer at pH 6.0 or 9.0, significant phospholipase A activity was observed qualitatively on thin-layer chromatograms by large increases in fatty acids and lysolecithin with a concomitant decrease in lecithin within 2 h. If the incubations were carried out under the conditions described by MARPLES AND THOMPSON[1], or by the preparative method of HANAHAN, RODBELL AND TURNER[3], or when lecithin is in excess of lysolecithin (3 to 1 mole ratio), very little residual activity in the recrystallized lysolecithin could be observed either by thin-layer chromatography or by fatty acid determinations, because the methods were not sufficiently sensitive to demonstrate the small amount of phospholipase A remaining. When incubated for 24 h as above in equal molar concentrations, no hydrolysis of lecithin occurred with lysolecithin prepared by the sodium methoxide method of MARINETTI[4].

When the above lysolecithin preparations were treated with trypsin and subsequently isolated, they did not exhibit any residual phospholipase activity after 24 h incubation with equal molar concentration of lecithin. Recovery of these preparations

was quantitative as compared to about 15–35% recovery of lysolecithin prepared by the sodium methoxide method[4].

A brief outline of further purification of lysolecithin prepared by the snake venom treatment of ovolecithin is given below:

Lysolecithin is prepared by the method of HANAHAN, BROCKERHOFF AND BARRON[5], and crystallized once from hot ethanol[2]. The crystalline lysolecithin is dissolved in a minimal amount of chloroform–methanol (1:1) and then evaporated to dryness. The residue is redissolved in small amounts of 0.1 M Tris buffer (pH 6.8) containing 1–5 mg crystalline trypsin (EC 3.4.4.4) (Armour), and 0.001 M $CaCl_2$. After incubation at 37° for 2.5–3 h, trypsin is inactivated by the addition of stoichiometric amounts of diisopropylfluorophosphate. Ten volumes of ethanol are then added to the mixture, the mixture warmed in a steam bath and filtered. The clear filtrate is evaporated to dryness and the residue is redissolved in chloroform–methanol (7:3) for subsequent chromatography on silicic acid. Chromatographically pure lysolecithin is eluted from this column with methanol.

The residual phospholipase activity in the non-trypsin treated lysolecithin preparation is not removed either on silicic acid or DEAE-cellulose columns.

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