磷脂酶A2 来源于东部菱背响尾蛇毒液

Method

Activity values for phospholipase A2 preparations which are derived from titrimetric assay procedures can be quite dependent on source and type of lecithin, its preparation as a substrate emulsion, other components of the reaction mixture, and the methodology/instrumentation utilized. Values reported in the literature for highly purified venom phospholipase A2 have ranged from 200 to 3000 units/mg. Comparison between assay systems is difficult. The following assay has been found to be reproducible in our laboratory. One unit releases one micromole titratable fatty acid per minute from lecithin emulsion at pH 8.9 and 25°C under the specified conditions.

Reagents

0.1 M Calcium chloride

1.0 M Sodium chloride

Lecithin emulsion: Weigh out 4.0 grams reagent grade soybean lecithin in a 250 ml beaker and add 30 ml of 1.0 M sodium chloride, 10 ml of 0.1 M calcium chloride and 100 ml of reagent grade water. After stirring for 30 minutes at 4°C, sonicate the mixture for 10 minutes at maximum power on a Branson sonicator or its equivalent. Dilute with reagent grade water to a final volume of 200 ml.

0.01-0.02 N NaOH - Standardized

Enzyme

Dissolve enzyme at a concentration of 1.0 mg/ml in reagent grade water. Keep this stock solution on ice while assay is being run. Further dilutions are made in reagent grade water immediately prior to use.

Procedure

The titration can he carried out with an automatic titrator or on a laboratory pH meter. The reaction vessel should be maintained at 25°C.

Blank rate determination: Pipette 15 ml of lecithin emulsion into a reaction vessel at 25°C. Adjust the pH to 8.9 and record the volume of titrant required to maintain the pH at

8.9 for 3-5 minutes after a constant rate is obtained. Determine the "blank rate" as the volume of titrant added per minute from the final linear portion of the curve.

Sample determination: Add appropriately diluted enzyme to the above emulsion. Record the amount of titrant required to maintain the pH at 8.9 for 4-5 minutes. Determine the "sample rate" as the volume of titrant added per minute from the linear portion of the curve.

Calculation

$$\frac{\text{Units}}{\text{mg}} = \frac{(\text{sample rate - blank rate}) \text{ x normaility of base x } 1000}{\text{mg enzyme in reaction mixture}}$$