IMPROVEMENT OF AN INEXPENSIVE METHOD FOR PHOSPHOLIPASE A_2 ACTIVITY DETERMINATION.

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Phospholipase A_2 (PLA_2) catalyses the hydrolysis of the 2-acyl ester bond of sn-2 phospholipids to give a long chain fatty acid and the corresponding lysophospholipid. Several methods exist to assay this activity, however, many of these methods employ expensive and sophisticated equipment. In this abstract, we describe an inexpensive, sensitive and reproducible assay to measure PLA_2 activity. Briefly, 0.3 ml of 4 x washed fresh mice erythrocytes were added to 0.3 ml of egg yolk dissolved 1:4 in 0.15 M NaCl and 0.25 ml of 0.1 M $CaCl_2$. This mixture was then added to 24.15 ml of 0.8% agarose in phosphate buffered saline (pH 7.4). The resulting solution was then poured on 84x89 mm glass plates (12 ml/plate) and allowed to gel. Nine 2mm wells were then punched isometrically on the gel and filled with 10 µm of serial dilutions of Crotalus durissus terrificus PLA_2 standard (Sigma Chemical Company). The plates were then incubated for 20 h at 37°C. PLA_2 activity was then assessed as a function of the hemolytic haloes. All the assays were made in triplicate.

Table 1-PLA_2 activity curve

PLA_2 (µg/ml)	0.24	0.46	0.93	1.87	3.75	7.5	15	30
Ø of the halo(cm)	0.80	1.00	1.20	1.30	1.35	1.40	1.50	1.60

From these data, we can conclude that the assay is highly sensitive and reproducible (no standard deviation was observed between the triplicates). The optimal sensitivity is reached between 0.24 and 1.87 µg/ml, where the curve behaves in a linear manner. This method is actually in use in our laboratory for purification screening as well as neutralization assays.

Supported by CNPq and CAPES