Direct determination of phospholipase D activity by infrared spectroscopy

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To determine phospholipase D (PLD) activity an infrared spectroscopy assay was developed, based on the phosphate vibrational mode of the phospholipid substrates of the enzyme such as dimyristoylphophatidylcholine (DMPC), lysophosphatidylglycerol (lysoPG) dimyristoylethanolamine (DMPE), and lysophosphatidylserine (lysoPS). Characteristic vibrational bands were located at 1230, 1226, 1221 and 1218 cm^{-1}, respectively, and served to monitor the hydrolysis of phospholipids. The appearance of the phosphate vibrational band of phosphatidate at 1130 cm^{-1}, served to monitor the amount of byproduct of the hydrolytic cleavage of phospholipids by PLD. *In situ* measurements could be performed within less than 20 min, using 2-40 mM DMPC and at least 5-10 ng of *S. chromofucus* PLD having specific activity of 30 nmol min^{-1} μg^{-1} (corresponding to 150- 300 pmol hydrolysed DMPC per minute) at pH 8.0 in the presence of 10 mM Ca^{2+}. The feasibility of the infrared assay using lysoPG, DMPE and lysoPS was also demonstrated, indicating that various natural phospholipids could be employed as substrates to measure the PLD activity. Reproducible apparent maximum velocities (V_{max}) were also determined. The direct infrared assay could be used as a possible screening tool to find specific PLD inhibitors.

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