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Determination of the Catalytic Activity of Phospholipase A_2: *E. coli*-Based Assay Compared to a Photometric Micelle Assay

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Dedicated to Prof. Dr. R. Kattermann on the occasion of his 60th birthday

Summary: Phospholipase A_2 activity in human sera was determined of the basis of the *E. coli* assay and compared to a photometric micelle assay. The *E. coli* assay is based on the hydrolysis of phospholipids from [1-^{14}C]oleic acid-labelled *E. coli* biomembranes. In the photometric assay the phospholipase A_2 acts on mixed phospholipid micelles. The amount of fatty acid produced is quantitated in a subsequent photometric assay by coupling in the reaction to the coenzyme A metabolism. The *E. coli* membranes are essentially resistent to other lipases in human sera, i. e. lipoprotein lipases, hepatic triacylglycerolipase or pancreatic lipase and thus a very specific substrate for the phospholipase A_2 of human serum. The photometric assay, though, is susceptible to other lipases in human serum. The ratio of [1-^{14}C]oleic acid to released total fatty acids served as the basis for the calculation of the true enzymatic activity. The assay closely correlated with the photometric assay based on mixed micelles in the higher ranges of phospholipase A_2 activity, but not in the normal range. The sensitivity is higher by at least two powers of 10. The human serum phospholipase A_2 strongly preferred *E. coli* membranes as substrate to the mixed micelles containing phosphatidylcholine/phosphatidylethanolamine. In conclusion, the modified phospholipase A_2 assay based on *E. coli* membranes is a sensitive, specific, reliable, and convenient method for the measurement of phospholipase A_2 activity in human sera. The photometric assay suffers from low sensitivity but has the advantage of practicability in a normal routine laboratory, including the amenability to automation.

Introduction

Various efforts have been made to develop sensitive assays for the determination of phospholipase A_2[1]) (1–16). The choice of a detection method depends partly on the goal of a particular experiment. For example, some assays can be used on purified enzymes but are incompatible with crude systems, some methods provide a continuous assay and generate a time course while others do not, and some methods are amenable to automation while others are not. However, the most important consideration in the choice of the detection method is the sensitivity required for the particular enzyme. The required sensitivity depends on the quantity of enzyme available and on its specific activity. This point is especially important for the assay of non-pancreatic phospholipases A_2 in human plasma, which are found in lower quantities and are, in general, less active than their counterparts from the pancreas or venom.

The sensitivity of an assay is influenced by a number of factors, the most important of which is the detection limit of a particular method. The detection limit

[1]) Enzymes
Phospholipase A_2 = phosphatide 2-acylhydrolase (EC 3.1.1.4)
Phospholipase A_1 = phosphatide 1-acylhydrolase (EC 3.1.1.32)
Lipase = triacylglycerol acylhydrolase (EC 3.1.1.3)