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Development of continuous and high throughput assay for measuring lipase and phospholipase activities and/or their inhibitors for applications in the field of health

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In human lipid metabolism, lipolytic enzymes such as lipases and phospholipases play a key role, as they degrade dietary as well as stored triglycerides (TGs) and phospholipids thus initiate and regulate the release of free fatty acids into the serum. These enzymes are therefore promising targets for the development of new drugs in the fields of obesity, diabetes and atherosclerosis. To date, several sensitive methods, based on radiolabeled elements or sterically hindered fluorochrome groups are usually employed to screen lipase or phospholipase A (PLA) activities. The purpose of this study is the development of a new ultraviolet spectrophotometric assay for lipase and PLA using TGs or phospholipids containing α-eleostearic acid (9Z, 11E, 13E-octadecatrienoic acid) and which were coated in the wells of microtiter plates. The conjugated triene present in α-eleostearic acid constitutes an intrinsic chromophore, which confers strong UV absorption properties on both the free fatty acid and the lipid substrate. Upon lipase or PLA action at the oil/water interface, α-eleostearic acid is released and desorbed from the interface and then solubilized into the micellar phase containing β-cyclodextrin (β-CD). Consequently, the UV absorbance is considerably enhanced due to its transition from the adsorbed state to the soluble state. This assay is based on the difference between the apparent molar extinction coefficients of the two types of α-eleostearic acid either esterified into lipid substrates or free into the reaction medium. The enzyme activity can be measured continuously by recording the variations with time of the UV absorption spectra. Low concentrations, down to 1 pg/mL of human pancreatic lipase or PLA2 could be detected under standard assay conditions. The detection sensitivity of this coated method is around 1000 times higher as compared to those obtained with the classical emulsified systems. This continuous high throughput assay could be used to screen new lipases/PLA or their inhibitors present in various biological samples.

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