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Fluorometric Method to Assess Lipase Inhibition Activity

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Abstract: Obesity and excess weight have become serious health problems in our developed societies today. Increased blood pressure, blood glucose levels and abnormal blood lipids are frequent consequences. Inhibition of digestive enzymes by pharmacological or nutritional intervention are one avenue to be considered to treat this population. In the present study a robust assay to screen biologically active materials for their ability to inhibit pancreatic lipase, the most important enzyme in fat digestion, has been developed. Methyl-umbelliferyl butyrate was used as an artificial substrate, enabling assessment of lipase activity *via* specific fluorescence emission. Applicability of the assay was shown by assessment of lipase inhibition activity of wild plants from Switzerland and France. Testing showed some plants to have a high inhibition rate of about 70%. In further projects, this lipase inhibition assay could be used for a scientific proof of biological activity of raw materials with the intention to develop functional foods for weight reduction.

Keywords: Digestion · Fluorometric assay · Lipase inhibition · Obesity

1. Introduction

Obesity and overweight are pertinent health problems in our developed societies. Increased blood pressure, blood glucose levels and abnormal blood lipids are frequent consequences. Billions are spent each year to provide treatments or appropriate technical assistance to people suffering from these consequences, also known as metabolic syndrome.[1] These conditions may be treated by various ways, including surgical interventions or pharmaceutical therapy with molecules that reduce the appetite or inhibit enzymes.[2–5] One of the targeted enzymes is pancreatic lipase. Pancreatic lipase is an esterase that cuts triacylglycerols into smaller size molecules (mono-glycerides and fatty acids).[6] This cleavage is the first step in the absorption process of triacylglycerols.[7] It is common knowledge that triacylglycerols contain energy in a condensed form, at a twofold higher level than found in proteins or carbohydrates. Consequently, inhibition of pancreatic lipase will result in a dramatic decrease of fat and energy intake.[8] Inhibition of pancreatic lipase is always incomplete, even if very potent inhibitors are administered.[4,9] Since this enzyme has an extremely high cleavage activity, sufficient cleaved triacylglycerols always remain to cover nutritional needs.[10] Inhibition of pancreatic lipase, the central fat digestion enzyme, will disturb fat assimilation and has the potential to help people manage their body weight.

The aim of the present study was to develop a robust and convenient assay, resembling physiological conditions, to screen extracts and isolated bioactive compounds for their ability to inhibit pancreatic lipase. This study is a preliminary study to show the method's feasibility and general applicability. In further projects, this method could be used for a scientific proof of biological activity of raw materials with the intention to develop functional foods for weight reduction.

2. Experimental

2.1 *Plant Material*

Wild plants from Switzerland and France were collected in April and May 2008, dried in a dry rack at Engineer school of Lullier in Geneva until constant weight (35 °C, 48 h, Salvis Incubator TSG 185, Emmenbrücke, Switzerland) and stored under dry conditions. Plants were directly used for digestion without further treatment.

Before digestion and for the DPPH assay, plants were crushed with a centrifugal grinding mill (Retsch ZM100, Haan, Germany) with a strainer diameter of 1 mm. For digestion, the well-homogenized, crushed material was used directly. To assay DPPH, 0.1 g of crunched plant material were extracted for 1 h with 1 mL of an ethanol water solution (20 + 80).

2.2 *Digestion Method*

Digestion was based on a published method.[11] Briefly, 0.1 g of crushed plant material was added to 12 mL of HCl (0.1 mol/L), and then the solution was heated to 37 °C in a water bath for 5 min. The pH was adjusted to 1.9 with NaOH (1 mol/L) and 2 mL of a pepsin solution (7 g/L pepsin in 0.1 mol/L HCl) was added. After 1 h, the digestion was stopped by increasing the pH to 7.4 using NaOH (1 mol/L). Following this step, 0.8 mL of a $NaHCO_3$ (3.4 g/L) solution and 2 mL of pancreatin solution (7 g/L pancreatin and 7.4 g/L bile salt, in saline buffer containing 135 mmol/L NaCl, 20 mmol/L $NaHCO_3$ at pH 7.4) were added to the reaction mixture. After 1 h of stirring at 37 °C, the solution was centrifuged before fluorometric assay.

2.3 *Fluorometric Assay*

The method was based on numerous works[12–16] adapted to comply with microwell plates suitable for enzyme inhibition activity assays. A substrate solution was prepared in a 1.5 mL Eppendorf by solubilisation of 6.0 mg of 4-methylumbelliferyl butyrate in 1000 µL of DMSO. Substrate was diluted fivefold with DMSO before analysis. Analyses were performed on microwell plates (NUNC Microwell 96 wells, black, Roskilde, Denmark), 100 µL of the digested mixture and 200 µL of 50 mM phosphate buffer solution at pH 7.4 were added in each well. Immediately prior to measurement with the fluorescence spectrometer (Varian, Fluorescence Spectrometer Cary Eclipse, Grenoble, France), 50 µL of substrate solution were added. The fluorescence emitted at 445 nm after excitation at 365 nm was recorded immediately. The speed of fluorescence development is directly proportional to the product formation and therefore to the pancreatic lipase activity.

2.4 *2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay*

A slightly modified method of Alamanni and Cosu[17] was used. A decolorization of a stable radical (DPPH) was induced

by antioxidants of the sample. A volume of 10 mL of a DPPH (2,2-diphenyl-1-picrylhydrazyl, 0.1 mmol/L) solution in methanol was mixed with 0.1 mL of plant extract. After 30 min, the absorbance was measured at 517 nm (Biochrom Libra S12 Spectrometer) with Trolox as a reference compound. Antioxidant capacity is expressed in mg/L Trolox equivalents.

2.5 *Reagents and Standards*

Hydrochloric acid and sodium hydroxide were provided by Panreac, Montcada, Spain. Pepsin, DMSO, DPPH (2,2-diphenyl-1-picrylhydrazyl), bile salts (cholic acid/deoxycholic acid) and sodium bicarbonate were provided by Fluka (Sigma-Aldrich), Buchs, Switzerland. 4-Methylumbelliferyl butyrate was provided by Sigma-Aldrich, Buchs, Switzerland. Trolox™ ((±)-6-hydroxy -2,5,7,8-tetramethylchromane-2-carboxylic acid) was provided by Acros, Geel, Belgium. Methanol was provided by Brenntag, Mülheim, Germany and ethanol by Cochimy, Martigny, Switzerland. Orlistat (Xenical™) was provided by Hoffmann-La-Roche Ltd, Division Pharma, Basel, Switzerland.

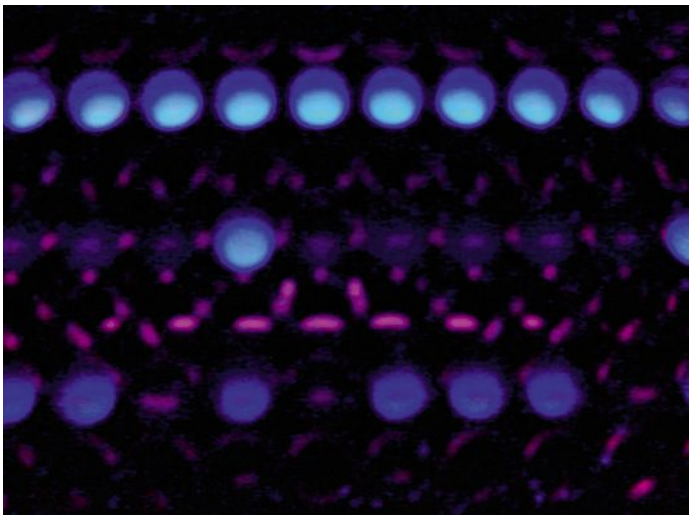

Fig. 1. Picture of a microwell plate showing fluorescence signals according to different inhibition induced by plant material.

3. Results and Discussion

A robust and convenient assay to screen biologically active materials for their ability to inhibit pancreatic lipase has been developed. Methyl-umbelliferyl butyrate was used as artificial substrate, enabling assessment of lipase activity *via* specific fluorescence emission. Applicability of this assay, close to the physiological conditions, has been tested with plant material and Xenical™ as a reference product. Tests with Xenical™ were made with and without digestion having no influence on the inhibition of lipase. A range of wild plants from Switzerland and France has been screened for activity to inhibit pancreatic lipase. For confidential purposes the names of the plants which served as test material are not mentioned. Some of these plants are under investigation as functional food ingredients for weight management. Influence of dose and linearity of response will be tested in these further experiments. A representative example of the selected plants having different inhibition activity is illustrated in Fig. 1.

The relative reaction speed of lipase is proportional to its activity in absence or presence of digested plant material and this is represented in Fig. 2. Some plants demonstrate a high inhibition rate of about 70%.

Under the selected test conditions, these plants exert a relatively high activity in the lipase inhibition assay compared to the inhibition activity of Xenical™/Orlistat. One explanation might be the enzyme's source for the test, porcine pancreatin, which is a mix of different enzymes and salts. Orlistat, which specifically inhibits cleavage of triacylglycerols by pancreatic and gastric lipase, might be ineffective on some esterases present in pancreatin, which might be able to convert the artifi-

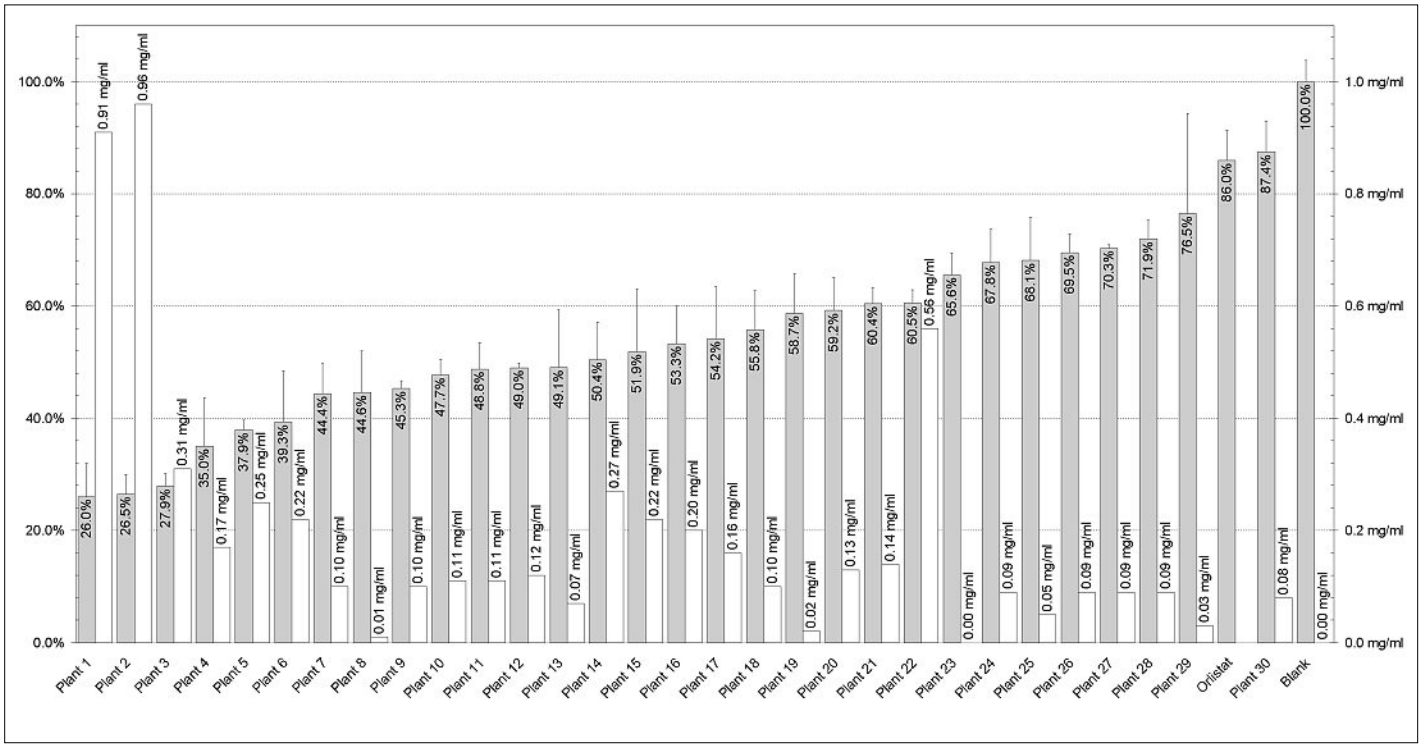

Fig. 2. Relative reaction speed of lipase with plants or Orlistat (grey bar, scale on the left) and antioxidant activity are given in Trolox equivalent (mg/mL) (white bar, scale on the right). Samples are sorted by their inhibition activity.

cial substrate. The plants provide a large spectrum of inhibition activity, far larger than the single compound Xenical™. With about 20% of activity reduction and the low amount of Xenical applied, nevertheless our assay confirms Xenical to be a potent lipase inhibitor.

Having a high affinity to proteins and peptide-like enzymes, polyphenolic compounds might influence the present lipase activity assay by non-specific binding. Therefore, polyphenol content of the plants was estimated by DPPH assay and values are also provided in Fig. 2. The most promising plants are those showing high lipase inhibition activity and low polyphenol content, such as plants 4 to plants 8 in Fig. 2.

This robust and convenient assay to screen biologically active extracts and isolated compounds is a helpful tool in development of functional foods for weight reduction. This assay enables scientific proof of inhibition activity of pancreatic lipase. Selection of promising extracts and ingredients as well as tracking each step of technological process for preservation of desired inhibition activity would be permitted by this valuable assay.

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