A MICROPLATE ASSAY FOR THE SCREENING OF *sn*-2 LIPASES

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Introduction. Lipases (EC 3.1.1.3) are lipolytic carboxylester hydrolases that catalyze the hydrolysis of the ester bonds of insoluble long-chain triacylglycerols (TAGs) releasing fatty acids; also they can hydrolyze a large variety of other carboxylic esters totally or partially insoluble in water. They are widely distributed in nature and have very attractive applications (1). Lipases have different specificities that make them a useful tool in biocatalysis. Most lipases can be classified as *sn*-1,3 regiospecific lipases, which hydrolyze acylglycerol only at the external positions of glycerol (*sn*-1 and *sn*-3), and nonregiospecific lipases, which act on all three positions of acylglycerols. Only a few lipases show a preference for the *sn*-2 position of acylglycerols (2). *sn*-2-specific lipases are particularly interesting for tailoring the fatty acids at the internal position of structured TAGs.
In the present work, we used a synthetic TAG containing α-eleostearic acid (E) as a chromophore only at *sn*-2 position and oleic acid (O) at *sn*-1 and *sn*-3 to establish an assay of lipases having specificity to hydrolyze the ester bond at the *sn-2* position, by comparing the hydrolysis of *sn*-2 substrate and tung oil TAGs (containing ≈70% of E).

Methods. The synthesis of 1,3-*O*-dioleoyl-2-*O*-α-eleostearoyl-*sn*-glycerol (OEO) (Fig. 1A) was conducted with pure and fully characterized reactants (1,3-*sn*-diolein and α-eleostearic acid) by Steglich sterification (3). Microtiter plate wells were coated with OEO and tung oil described by Verger et al. (5) and left to equilibrate at 25°C for at least 10 min with 200 µL of the assay buffer (10 mM Tris-HCl buffer, pH 8.0 containing 150 mM NaCl, 6 mM $CaCl_2$, 1 mM EDTA, and 3mg/ml β-CD). A lipase solution (2–10 µl) was added to each well, and the optical density (OD) at 272 nm was recorded continuously at regular time intervals of 30 s against the buffer alone using a microtiter plate-scanning spectrophotometer. Lipase A from *Candida antarctica* (CALA) was a generous gift from Dr. S. Patkar (Novozymes, Denmark). LIP2 lipase from *Yarrowia lipolytica* (YLLIP2) was produced and purified according (4). *Candida rugosa* lipase (CRL) AY30 was purchased from Amano Pharmaceuticals Ltd.

Results. The λ Max of α-eleostearic acid (272nm) was determined by spectra in the assay conditions and a similar spectra was obtained to OEO; at this λ the oleic acid showed no absorbance (Fig. 1B). The addition α-eleostearic acid at buffer in the reaction conditions increased the OD at 272 nm (Fig. 2A). Lipases that are known to have different stereoselectivities showed similar velocities to hydrolyze tung oil and different velocities to release (CALA and CRL AY30) or not (YLLIP2) the E sterified in the *sn*-2 position of OEO (Fig. 2B, C and D).

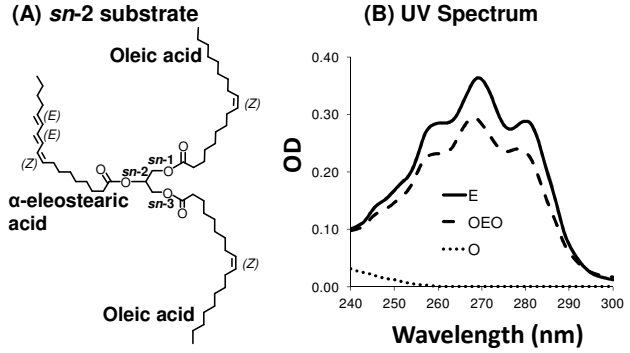

Fig.1 A) *sn*-2 substrate (OEO). **B)** Ultraviolet absorption spectrum of α-eleostearic acid (E), oleic acid (O) and OEO.

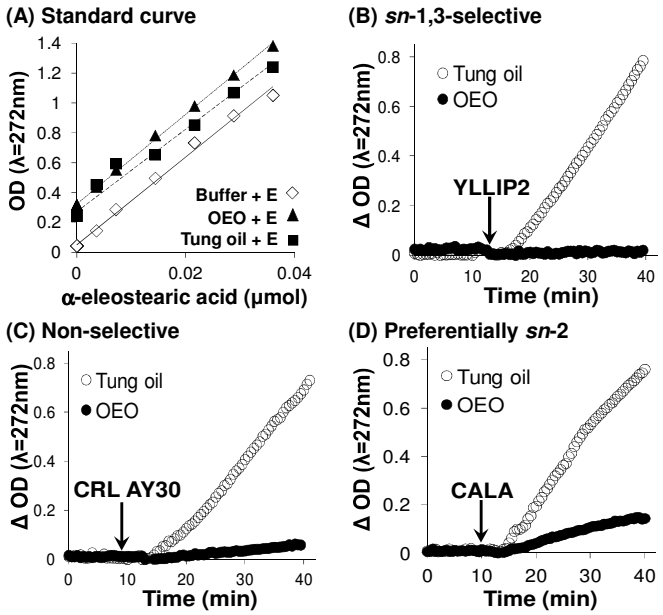

Fig.2 A) Standard curve of α-eleostearic acid (E) at 272 nm recorded in the reaction conditions. **B), C)** and **D)** Assays of the lipase activities using TAGs from tung oil and *sn*-2 substrate (OEO).

Conclusions. The synthetic OEO substrate appears to be a convenient tool for studying the *sn*-2 position specificity of lipases in microplate by UV detection taking in account the ratio of initial reaction rate of OEO and tung oil hydrolysis.

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