DEVELOPMENT OF A SCREENING ASSAY FOR A LIPASE USING SCINTILLATION AND IMAGING PROXIMITY ASSAY TECHNOLOGIES

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Introduction

A screening assay has been developed for a lipase enzyme using the Scintillation Proximity Assay (SPA) technology and LEADseekerä Homogeneous Imaging System. The assay uses a novel [^{3}H] labelled biotinylated lipid substrate which was presented to the enzyme bound to the surface of SPA and LEADseeker beads. The use of lipid coated beads produced an artificial membrane environment suitable for enzyme activity.

Initial assay development was carried out using a 96-well SPA assay format before miniaturization to 384-well format for imaging using LEADseeker. Both assay formats were used in inhibition studies with Ebelactone B.

Method

96-well SPA format. Using an 'on bead' assay format, 180nM lipase substrate was added to streptavidin coated yttrium silicate (YSi) beads @ 5mg/ml and 10% (v/v) Tritonä X-100 in the ratio of 1:3:2. Triton X-100 was added to prevent non-specific binding of the tritiated product to beads. Assays contained 20µl substrate precoated SPA beads (6nM lipase substrate, 50µg beads, 0.67% (v/v) Triton X-100), 5nM lipase and assay buffer (50mM Hepes, pH 7.5, 1mM dithioerythritol (DTE) and 0.001% (v/v) C13E12) in a volume of 100µl. In 'no enzyme' controls, approximately 15 000 SPA cpm were obtained. After incubation for 60 minutes at room temperature with agitation, assays were stopped by the addition of 100µl 0.1M sodium citrate/citric acid, pH 4.0 and counted on a TopCount™ microplate scintilliation counter.

384-well SPA and LEADseeker formats. 180nM lipase was added to streptavidin coated YSi beads @ 10mg/ml and 10% (v/v) Tritonä X-100 in the ratio of 2:3:1. Assays contained 10µl substrate precoated streptavidin YSi SPA beads or streptavidin yttrium oxide (YOx) LEADseeker beads (24nM lipase substrate, 50µg beads, 0.67% (v/v) Triton X-100), 30nM lipase and assay buffer in a volume of 25µl. In 'no enzyme' controls, approximately 6 000 SPA cpm and 600 IOD's were obtained. After incubation for 60 minutes at room temperature with agitation, assays were stopped by the addition of 25µl 0.1M sodium citrate/citric acid, pH 4.0 and counted on either on a TopCount microplate scintilliation counter or imaged on LEADseeker.

Results

Development of a lipase assay using a 96-well SPA assay format. Enzyme titrations were set up using 'on' and 'off' bead assay formats. In the 'off' bead format, the lipase substrate was not precoated onto SPA beads, instead the beads were added at the same time as the stop solution. Using an 'on' bead assay format resulted in a higher percentage of substrate cleavage than using an 'off' bead format and a more reproducible assay, as seen by the tighter error bars. It is thought that the binding of the substrate to the bead surface produced an artificial membrane environment necessary for optimal enzyme activity (Figure 1).

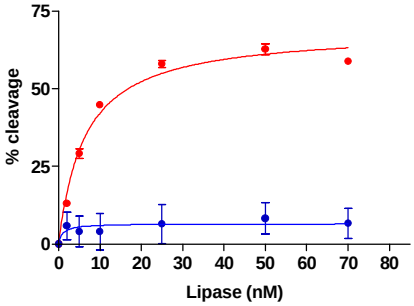

Figure 1. Enzyme titration comparing 'on' (·) and 'off' (·) bead assay formats.
Each data point in this and subsequent figures is the mean (± SEM) of 3 replicates.

The maximum substrate cleavage obtained was approximately 60%. Due to the nature of the 'on' bead assay, it is unlikely that 100% substrate cleavage would be obtained since steric hindrance, caused by the presence of the bead, is likely to affect enzyme activity.

Using the 'on' bead assay format, a time course was established using 5nM lipase (Figure 2).

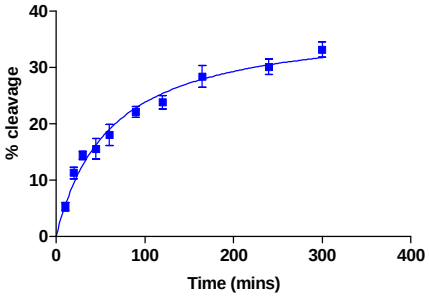

Figure 2. The effect of time on substrate cleavage by 5nM lipase.

Ebelactone B, a natural product from *Streptomyces aburaviensis*, is a known inhibitor of lipase activity[1]. Inhibition studies were carried out using Ebelactone B and an IC_{50} value of 27.5µM was obtained (Figure 3).

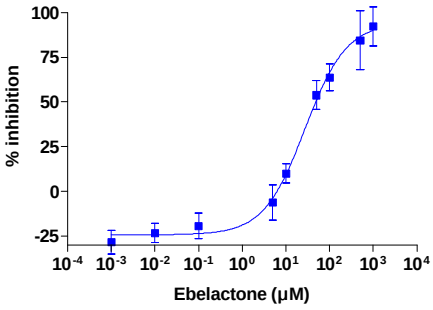

Figure 3. Inhibition of lipase activity by Ebelactone B. 5nM lipase was used and assays were incubated at room temperature for 60 minutes.

Miniaturization of the lipase assay to 384-well format using SPA and LEADseeker. Using a reduced assay volume an enzyme titration was performed with YSi SPA beads and YOx LEADseeker beads (Figure 4).

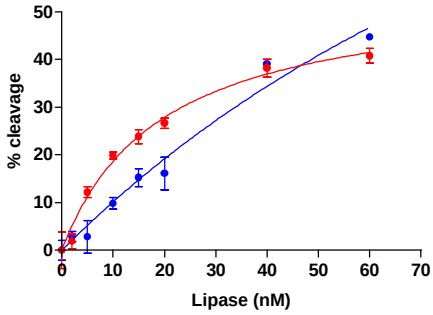

Figure 4. Enzyme titration comparing SPA (·) and LEADseeker (·) assay formats.

Different profiles were obtained using the two assay formats and this may be a result of how the substrate is presented on the surface of the two bead types.

Inhibition curves were obtained for the miniaturized assays using Ebelactone B and IC_{50} values of 32.3µM and 18.2µM were determined using SPA and LEADseeker formats, respectively (Figure 5). These values are comparable to the one calculated using the 96-well SPA assay format.

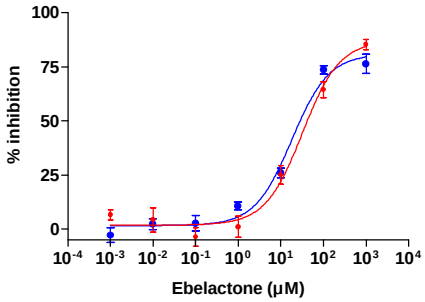

Figure 5. Inhibition of lipase activity by Ebelactone B detected using YSi SPA beads (·) and YOx LEADseeker beads (·). 30nM lipase was used and assays were incubated at room temperature for 60 minutes.

CONCLUSIONS

- A homogeneous assay has been developed using the Scintillation Proximity Assay technology and LEADseeker Homogeneous Imaging System suitable for measuring lipase activity.
- Assays are carried out using an 'on' bead assay format, creating an artificial membrane environment optimal for enzyme activity.
- The assay is suitable for use with both SPA, using 96 or 384-well formats, and LEADseeker, using a 384-well format.
- Lipase activity was inhibited by Ebelactone B and comparable IC_{50} values were obtained using the different assay formats.

Reference

1. NONAKA, Y. *et al*., *J. Enzyme Inhibition* **10**, pp57-63 (1995).

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