AGONIST AND ANTAGONIST ACTIVITY IN A GFP YEAST BASED ESTROGEN RECEPTOR FUNCTIONAL ASSAY

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ABSTRACT

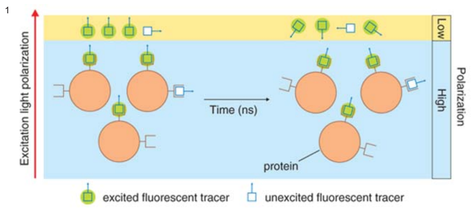
Estrogen receptors (ERα and ERβ) are ligand-binding transcription factors activated by the hormone 17-β estradiol. Ligand binding triggers ER dimerization, translocation of the receptor from the cytosol into the nucleus and eventually activation of the genes under control of ER. Studies have revealed a role for estrogen receptors in male and female sexual development, reproductive functions, bone metabolism and regulation of neuroendocrine and cardiovascular systems. ER is also known to bind to other non-native ligands known in pharmacology as receptor agonists or antagonists. Agonists provoke a biological response when bound to the receptor; antagonists inhibit a biological response when bound. Our lab is interested in the promiscuous binding of the estrogen receptor and its ability to activate different genes in different tissues. Fluorescence polarization assays have previously been performed using ER to study ligand binding affinities for the receptor. However, this technique is unable to determine whether these ligands are agonists or antagonists and allow ER dimerization and gene activation. To investigate these phenomena, an activity assay that measures ER controlled gene expression has been developed which provides the opportunity to gain further insight into the functional activity in living systems. Recombinant yeast cells that express ERα use the green fluorescent protein (GFP) reporter to determine whether ERα, in the presence of a particular ligand, has activated gene of expression. We have correlated the binding to agonist and antagonist behavior of several xeno- and phyto- estrogens.

INTRODUCTION

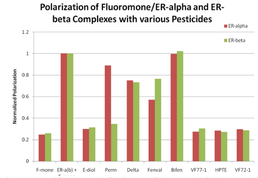
Competitive Ligand Binding Assay

Invitrogen fluorescence polarization assays use fluormone™, a fluorescent estrogen ligand with low polarization

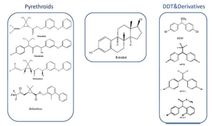
- Addition of ER to fluormone™ results in a bound complex and high polarization
- Addition of ER/fluormone™ complex to competitor molecules results in low polarization if the competitor displaces the fluormone™
- The change in polarization in the presence of a competitor molecule is used to determine the binding affinities of test compounds of interest



RESULTS AND LIMITATIONS



Data courtesy of Suzannah Luft, Eric Glustrom, Elias Aba-Miliki, Richmond Ampiah-Bonney and Patricia O'Hara

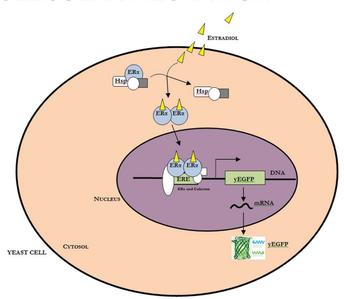


- Competitive ligand binding assays allow us to measure the relative binding affinities of several non-native ligands to ER
- For example, some DDT derivatives such as HPTE, VF77-1 and Vf72-1 were found to bind strongly to ERα and ERβ
- However, these binding assays do not allow us to determine ER-mediated gene activation and therefore do not distinguish between agonists and antagonists

SOLUTION

The yeast estrogen bioassay developed by Dr. Toine Bovee et al. in the Netherlands[1] provides a solution to this problem. In the bioassay, mutant *S. cerevisiae* express human ERα (hERα) and use the yeast enhanced green fluorescent protein (yEGFP) reporter construct. yEGFP exhibits bright green fluorescence (λ_{em}=530 nm) when exposed to violet light (λ_{ex}=488 nm)

yEGFP MOLECULAR MECHANISM



- Estradiol enters the yeast cell, binds ERα and inhibitory chaperone proteins dissociate
- ERα dimerizes and translocates into the nucleus where the ERα homodimer binds EREs within the yEGFP promoter. Cofactors are recruited to activate transcription of yEGFP
- yEGFP fluorescence can be used to determine whether a ligand of interest has activated yEGFP transcription
- Fluorescence intensity can also be used to determine the extent of ERα activation

METHOD AND RESULTS

Storage Conditions

- Inoculate agar plate containing selective medium with yeast from frozen -80 °C glycerol stock (20% glycerol v/v). Incubate plate at 30°C for 24-48 hours and store at 4°C for 2 weeks

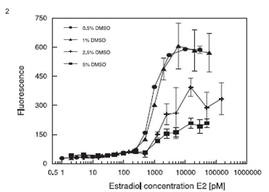
Culture Conditions

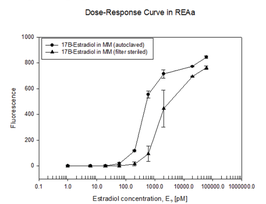
- Selective minimal medium containsYNB (-AA/-AS), dextrose, ammonium sulfate and L-leucine (MM_L)

yEGFP Assay

- Use one colony to incolulate 10 mL MM_L. Leave culture to grow overnight at 30°C and 200 rpm
- Dilute overnight culture in fresh MM_L such that the OD_{630}=0.05 ± 0.01.
- Add 200 µL of diluted culture and 2 µL of test compounds dissolved in DMSO to 96 well microplate. Incubate for 4-24 hours at 30°C and 200 rpm
- Measure fluorescence using The SpectraMax® M5 Multi-Detection Microplate Reader. Excite at 488 nm and measure emission at 530 nm

DOSE-RESPONSE CURVES





CHALLENGES AND FUTURE WORK

- Somewhat difficult to obtain suitable dose-response curves using the native estradiol ligand
- Optimize *S. cerevisiae* bioassay
- Use yEGFP bioassay to identify the agonist and antagonist behavior of previously characterized ligands
- Use assay to investigate other compounds of interest in the O'Hara Lab

ACKNOWLEDGEMENTS

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LITERATURE CITED

[1] http://www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook/Technical-Notes-and-Product-Highlights/Fluorescence-Polarization-FP.html
[2] Bovee, Toine F. et al., "Development of A Yeast Estrogen Bioassay, Based on the Expression of Green Fluorescent Protein," Gene 325 (Feb 2004):187-200