Abstract

The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) created by the Environmental Protection Agency was mandated with developing methods to screen approximately 87,000 chemicals for biological effects on estrogen, androgen and thyroid hormone systems. As part of this mandate, EDSTAC proposed that EPA develop rapid, high throughput screening systems to assess a compound’s effects on hormonal systems. The Center for Environmental Biotechnology at the University of Tennessee has re-engineered the Saccharomyces cerevisiae BLYES colorimetric estrogen reporter system (Routledge and Sumpter, 1996) to produce bioluminescence in response to estrogen or environmental estrogens (S. cerevisiae BLYES; Figure 1; Gupta et al., 2003). Bioluminescence is a reagentless system eliminating the need for expensive chromophores. Light detection is more sensitive than absorbance detection thus shortening the development time of the assay.

In previous work, strain BLYES was exposed to the estrogenic compounds 17β-estradiol, 17α-estradiol, 17α-ethyl estradiol, estrone, and 3,4,5-trichloro-4-biphenylol and compared to the YES assay (Fig. 2). The EC50 values correlated linearly (R2=0.97) between the 2 assays. Sensitivities of both assays decreased in the order 17β-estradiol > 17α-ethyl estradiol - estradiol = estrone > 17α-estradiol, with no significant response generated from 3,4,5-trichloro-4-biphenylol where the hydroxyl group is the sterically hindered by the paired ortho chlorines. The BLYES screen consistently detected estrogenic potencies at 5- to 10-fold lower levels than those attained in the YES assay. Moreover, bioluminescence was detectable in less than 4 hours as compared to 3 days for the colorimetric YES strain.

The primary objective of this research is to validate the BLYES system and develop a standard operating procedure for routine chemical analysis. Work is in progress to test the S. cerevisiae BLYES using the proposed 78 substances (ICCVAM, 2002) listed for validation of estrogen receptors and correlate to the colorimetric S. cerevisiae YES assay. Parallel research includes developing the S. cerevisiae BLYES into a standard assay suitable for HTS of chemicals and to modify the lacZ genes for optimum transcription/translation in S. cerevisiae thus increasing sensitivity of the assay.

Impact

This EPA-sponsored research seeks to standardize a bioluminescent yeast assay for screening substances that potentially interact with the human estrogen receptor. The bioluminescent reporter system has several advantages including:

• A low-cost, reagentless reporter system that eliminates the extra manipulation and cost of adding exogenous reagents.

• Speed; preliminary data using 17β-estradiol indicated a bioluminescent response in 2-4 hours and a maximum response in 15 hours (Fig. 3).

• Amenable to automated, high throughput analysis, data collection and interpretation.

• Cells for the assay can be prepared fresh or stored at ~80°C.

• No animals are used in this assay.

• Elimination of labor-intensive cell culture assays.

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Literature Cited


Figure 1. Construction of the estrogen inducible bioreporter S. cerevisiae BLYES. Synthesis of lacZ is regulated on plasmid pEREAB by upstream incorporation of two sequential estrogen response elements (ERE) coupled to a human phosphoglycerate kinase (PGK) promoter. The luxD component of the luciferase is supplied via independent expression from a fused IRES. The luxC, luxD, luxE and frp genes are under control of constitutive promoters on the plasmid pLCIRESfrp. The human estrogen receptor (hER-α) is inserted in the S. cerevisiae chromosome.

Figure 2. EC50 dose response profiles of the S. cerevisiae BLYES bioreporter to the estrogenic compounds 17β-estradiol, 17α-ethyl estradiol, estrone, and 3,4,5-trichloro-4-biphenylol and compared to the YES assay (Fig. 2). The EC50 values correlated linearly (R2=0.97) between the 2 assays. Sensitivities of both assays decreased in the order 17β-estradiol > 17α-ethyl estradiol - estradiol = estrone > 17α-estradiol, with no significant response generated from 3,4,5-trichloro-4-biphenylol where the hydroxyl group is the sterically hindered by the paired ortho chlorines. The BLYES screen consistently detected estrogenic potencies at 5- to 10-fold lower levels than those attained in the YES assay. Moreover, bioluminescence was detectable in less than 4 hours as compared to 3 days for the colorimetric YES strain.

Figure 3. 3-Dimensional plot of bioluminescence vs. time for 0-62.5 ppb 17β-estradiol. Initial bioluminescence is observed in as little as 2 hours and peaks at approximately 15 h.