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In vitro bioactivity of 17α-estradiol

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Abstract

A miniaturised short-term in vitro assay based on the activation of the human estrogen receptor α and genetically modified yeast (*Saccharomyces cerevisiae*) cells was performed to explore the capacity of this system to monitor the bioactivity of estrogenic compounds, particularly 17α- and 17β-estradiol. Together with the human estrogen receptor (hER)-α plasmid, the reporter plasmid containing a yeast-optimised version of the green fluorescent protein (yEGFP) linked to three repeats of the *cis*-acting estrogen hormone-responsive element (ERE) were expressed in a strain being deleted in the pleiotropic drug resistance transporters Pdr5, Snq2 and Yor1, known to facilitate efflux of organic compounds including steroids and chemotherapeutics. Agonists that bind to hER in vitro trigger estrogen receptor-mediated transcriptional activation of the *GFP* reporter gene monitored by fluorescence emission at 535 nm. The sensitivity of the assay was tested with various 17α- and 17β-estradiol concentrations, yielding a detection limit of 5 pg/ml (0.018 nM) for the agonist 17β-E2 in solvent and in human charcoal-stripped serum using a *S. cerevisiae pdr5, snq2* and *yor1* mutant strain. For 17α-estradiol only, at approximately 1500 pg/ml a similar fluorescence response compared to 100 pg/ml 17β-E2 was observed implicating a much weaker potency of this stereoisomer. The specificity of the system was tested by expression of a truncated hER lacking the ligand-binding domain E and by administration of the androgen, 4-androsten 3,17 dione. Both controls did not yield an increase in fluorescence emission. This fluorescence emission assay enables detection of estrogenic biological activity induced by direct agonists, such as 17β-E2 at concentrations similar to those found in human sera or by estrogen-like chemicals.

1. Introduction

Estradiol, the most potent natural estrogen, occurs in two isomeric forms that differ in the steric position of the hydroxy-group on C17. In most mammalian species, 17β-E2 is the natural estrogen secreted by the ovaries and produced by peripheral tissues. 17α-E2, in contrast, is the predominant estrogen in some ungulates and rodents [1]. In humans, 17α-estradiol has only been found under some specific (rare) circumstances in the urine and serum [2]; it is generally considered as being devoid of classical biological estrogen activity. Nevertheless, the 17α-estradiol biological profile has not been systematically examined.

Saccharomyces cerevisiae tester strains have widely been developed for detection of estrogenic activity using growth on selective media [3] or β-galactosidase production and photometric determination of the conversion of chromogenic substrates as reporter [4–6]. However, cell extract preparation and the enzymatic conversion assay require manual work preceding the photometric measurements. Usage of the yeast-optimised green fluorescent protein (yEGFP) linked to the estrogen-responsive element (ERE) may thus provide a valuable tool for direct optical read-outs following exposure of cells to estrogens [7,8]. In addition, the influence of general mechanisms that confer tolerance to a great variety of compounds has so far received limited attention in the context of hormone activity testing [9]. In the yeast *S. cerevisiae*, a so-called pleiotropic drug resistance (PDR) network [10] comprises the major determinants of multiple drug resistance [11]. Induction by a drug or toxin involves the upregulation of expression of well-characterised genes that encode transporters involved in drug extrusion, and thus multidrug resistance. The majority of these proteins have

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broad overlapping specificities for compounds with very different chemical structures and cellular targets; transported compounds include a variety of anticancer drugs, antibiotics, antifungals, detergents, ionophores and steroids [9–14]. Potentially, the activities of these ABC-type extrusion systems confound attempts to develop bioassays for estrogens, since continuous removal of the relevant compound from the cell compromises the sensitivity of any assay. To enhance the sensitivity of the assay, the receptor and reporter constructs have been expressed in a yeast strain deleted in the multidrug ABC-transporter genes *PDR5* (*p*leiotropic *d*rug *r*esponse), *SNQ2* (disruption confers *s*ensitivity to 4-*n*itro*q*uinoline-N-oxide) and *YOR1* (*y*east *o*ligomycin *r*esistance). In conjunction with estrogenic activity detection, established growth assays were performed by measuring the optical density to normalise for specific fluorescence development. Using a miniaturised and partially automatised test system, dose–response curves were obtained for 17α- and 17β-estradiol in solvent and in the presence of human charcoal-stripped serum, for two phytoestrogens and one mycotoxin, estrogenic chemicals and the androgen, 4-androsten 3,17 dione.

2. Material and methods

2.1. Yeast strains and growth conditions

Haploid *S. cerevisiae* yeast strains used throughout this study are summarised in Table 1. All yeast cells were grown at 30 °C. Nutritional requirements appropriate for selection and maintenance of mutants and plasmids in the transformed strain were scored on minimal YNB media consisting of 0.67% yeast nitrogen base (YNB) with $(NH_4)_2SO_4$ and 0.5% carbon source (D-glucose) adjusted to pH 6.4 with 50 mM citric acid buffer and supplemented with the appropriate amino acids except tryptophan, leucine and the pyrimidine base uracil to utilise besides auxotrophic marker selection, the LEU2-d function of the pERE-CYC1-GFP vector to increase for plasmid copy numbers. The plasmids were obtained by standard DNA manipulations according to Sambrook et al. [15] and used to transform *S. cerevisiae* wild type and FYAK26/8-10B1 cells [13] to tryptophan, uracil and leucine prototrophy by standard methods [16]. All amino acids were purchased from Fluka, YNB from Difco and DMSO from Sigma–Aldrich. All components were of analytical quality. Plasmid-dependent phenotypes were regularly checked by plasmid loss experiments.

2.2. Plasmid construction

Plasmid YEpE12 was a gift from Dr. A. Jungbauer (University of Vienna). According to the confirmed hER sequence, the truncated version of the hER was constructed by using the single restriction sites *Nco* I and *Sac* I, eliminating 608 bp, and thus, most of the C-terminal ligand-binding domain E. Protruding ends were filled with T4-DNA-Polymerase (MBI Fermentas) and dNTPS and religated to plasmid YEpE12t608. The episomal vector backbone to construct the green-fluorescent protein reporter plasmid pERE-CYC1-GFP was the high copy *Escherichia coli*/yeast shuttle vector pYEX-BX (Clontech, Palo Alto, USA). The CUP I-promoter was first replaced by using the *Xba* I and *Bam*H I restriction sites and ligation to a cassette containing three repeats of the estrogen receptor-responsive element (5′-GGGTCACAGTGACCGCTAG-3′) with a CAG triplett as spacer between the palindromic sequences and containing primer-encoded *Xba* I and *Bam*H I restriction sites to pERE. A CYC1 minimal promotor (chromosome X, SGD database, Stanford) element of 259 bp was amplified from *S. cerevisiae* wild type genomic DNA as template by PCR with TAQ Polymerase (MBI Fermentas) using primers corresponding to the nucleotides 525779–525796 (SGD database) (5′-AGAGA-*GGATCC*CTCGAGCAGATCCGCCAG-3′) and 526028–526004 (5′-AGAGA*GTCGAC*TATTAATTTAGTGTGTGT-ATTTGTG-3′) containing primer-encoded *Bam*H I and *Sal* I restriction sites. The fragment was then ligated to the *Bam*H I and *Sal* I restriction sites of pERE. The yeast-enhanced *yEGFP* gene of *Aequorea victoria* [17] was inserted in pERE-CYC1 by ligation to *Sal* I and *Pst* I restrictions sites to yield pERE-CYC1-GFP. The recombinant plasmids, recovered from transformed *E. coli* XL1-blue cells were mapped by restriction analysis and confirmed by sequencing (GeneART). Computer analysis of nucleotide and amino acid sequences were performed using the Vnti software (Informax, UK).

2.3. Assay conditions and fluorescence monitoring

For quantitative assessment of growth phenotypes and fluorescence development logarithmic growing cells (70% budding) were diluted to a start OD_{600} of 0.4 (Pharmacia

Table 1
Haploid yeast strains

	Genotype	Source
FY1679-28C	*MATa, ura 3-52, trp 1Δ63, leu 2Δ1, his3Δ200, GAL2+*	Kolaczkowski et al., 1998
FYAK26/8-10B1	*MATa, ura 3-52, trp 1Δ63, leu 2Δ1, his3Δ200, GAL2+, pdr5-Δ1::hisG, snq2::hisG, yor1-1::hisG*	Kolaczkowski et al., 1998
ASY14	FYAK26/8-10B1 [pERE-CYC1-GFP]	This study
ASY15	FY1679-28C [pERE-CYC1-GFP] [YEpE12]	This study
ASY16	FYAK26/8-10B1 [pERE-CYC1-GFP] [YEpE12]	This study
ASY17	FY1679-28C [pERE-CYC1-GFP] [YEpE12 Δ 1184-1793]	This study
ASY18	FYAK26/8-10B1 [pERE-CYC1-GFP] [YEpE12 Δ 1184-1793]	This study

Ultrospec 2000 Spectrophotometer) corresponding to 3.25×10^6 cells/ml. For each tested compound, at least three replicate tests were carried out on different days. Each experiment consisted of four identical test cultures with one control and minimum four test concentrations with reporter induction scored between >0 and <100% biological activity. Test compounds were dissolved in DMSO or human charcoal-stripped serum and added to the test cultures of yeast strains transformed with the respective expression-reporter system in a total volume of 200 μl. The final concentration of the DMSO solvent did not exceed 0.5%. For serum samples, 10% human charcoal-stripped serum was used (provided by Dr. Daxenbichler, University of Innsbruck), the charcoal–dextran mixture is able to adsorb free and loosely bound hormone. The growth was estimated by OD_{600} measurements in 15 min intervals in transparent 96-well microtitre plates using a microplate reader (Tecan, Spectrofluor Plus). Tests were considered as valid when the turbidity of the control cultures increased at least five-fold during the incubation period. For fluorescence development read-outs, the excitation wavelength was adjusted to 485 nm and emission was observed at 535 (25 nm bandwidth) in 15 min intervals for 16.5 h. Dose–response curves were obtained according to: $FLUO/OD = (FLUO_{ci} - FLUO_{Bci})/(OD_{ci} - OD_{Bci})$, where the fluorescence at the test concentration (ci) minus the corresponding values for the blank control (Bci) is divided by growth determined as OD 600 (corrected for blanks). This is similar to the calculation of Miller units for β-galactosidase expression where the *o*-nitrophenol activity at A_{420} is normalised to cell density, time and volume of the probe except that in our method, corrections for pure medium and medium plus solvent fluorescence/OD controls are incorporated.

3. Results

Two expression-reporter systems were constructed in *S. cerevisiae* to explore hER-driven transcriptional activation of the green fluorescent protein as reporter to measure ligand-induced bioactivity. One system deployed *S. cerevisiae* wild type and in a second approach, aiming for an increased sensitive detection of potential estrogenic activity, a *S. cerevisiae* strain devoid of the pleiotropic drug transporters Pdr5, Snq2 and Yor1 [13] was used as host. The two-plasmid expression system produced the recombinant human estrogen receptor α that, upon ligand-mediated activiation, bound three copies of the human estrogen-responsive element (ERE) upstream of the yeast-enhanced version of the GFP of *A. Victoria* (yEGFP). The fluorescence development in the transformed mutant strain was compared to that of the corresponding *S. cerevisiae* wild type and to that of a strain expressing a C-terminal truncated, lacking most of the ligand-binding domain E, and thus non-functional version of the receptor. In the presence of 17β-E2, both *S. cerevisiae* wild type and the mutant indicator strain started to produce increasing amounts of GFP at approximately 2.8 h, which is almost equivalent to the progression of one full cell cycle, and about the time required for full functional folding of GFP (data not shown). During logarithmic growth phase, the cell number increased not proportionally to the net GFP production, such that the fluorescence emission developments finally entered a plateau phase at 14 h incubation. In contrast, those strains expressing the truncated hER exhibited net decreasing fluorescence/OD read-outs throughout the test period, meaning that no additionally induced GFP production besides the background occurred in the growing yeast cell population. Dose-dependent response curves were obtained from the Fluo/OD-values at the end of the 16.5 h incubation period. The dose–response relationship – fluorescence emission normalised to the simultaneously detected absorption at 600 nm – for different concentrations of 17β- and 17α-estradiol is given in Fig. 1. The minimal concentration of 17β-E2 at which *S. cerevisiae pdr5 snq2 yor1* cells started to respond was 5 pg/ml (0.018 nM) with an unusual, repetitive peak at 10 pg/ml (Fig. 1A). Upon exposure, the relative ERE-GFP-induced fluorescence signal correlated with increasing concentrations of the hormone up to 1000 pg/ml (Fig. 1C) in cells expressing the functional hER with an EC_{50} of 60 pg/ml (0.216 nM). Higher, non-physiological concentrations of 3000 pg/ml 17β-E2 did not yield further increasing light emissions indicating a possible saturation limit of the test system around 1000 pg/ml 17β-E2. No such dose-dependent response was observed upon 17α-E2 incubation within the same concentration range between 0 and 100 pg/ml (Fig. 1B). The similar effective response for 17α-E2 was observed at approximately 1500 pg/ml (Fig. 1C), and thus ∼15 times higher concentrations of 17α-E2 were necessary to yield a similar fluorescence emission signal intensity as with 17β-E2. Even with 3000 pg/ml 17α-E2, no saturation limit was observed. No dose-response was observed with the non-functional human estrogen receptor control strains (Fig. 1A and B). The androgen, 4-androsten 3,17 dione was tested as negative effector for the estrogen receptor, and thus fluorescence development, and even at 3000 pg/ml did not lead to detectable GFP production in cells expressing the functional hER (Fig. 2). The biological estrogenic activity of the phytoestrogens: Coumestrol, Genistin and the mycotoxin, Zearalenon were with EC_{50}-values of 700, 1200 and 1000 pg/ml (2.61, 4.44 and 3.14 nM), respectively, substantially lower than that of the true agonist 17β-E2 (Fig. 3A). Interestingly, the synthetic estrogen DES (Fig. 3B) revealed an EC_{50} of 400 pg/ml (1.44 nM). The estrogenic compounds ethinyl-estradiol and estradiol-3-benzoate (Fig. 4) exhibited qualitatively different responses, with ethinyl-estradiol as potent (EC_{50} 150 pg/ml, 0.51 nM) and estradiol-3-benzoate as very weak effector. Using up to 10,000 pg/ml, no saturation limit was determined.

To analyze the effects of extracellular proteins on the activity of 17β-E2, the indicator strains were cultured in medium containing charcoal-stripped human serum (Fig. 5A). At concentrations of 10% serum in the medium, the fluorescence

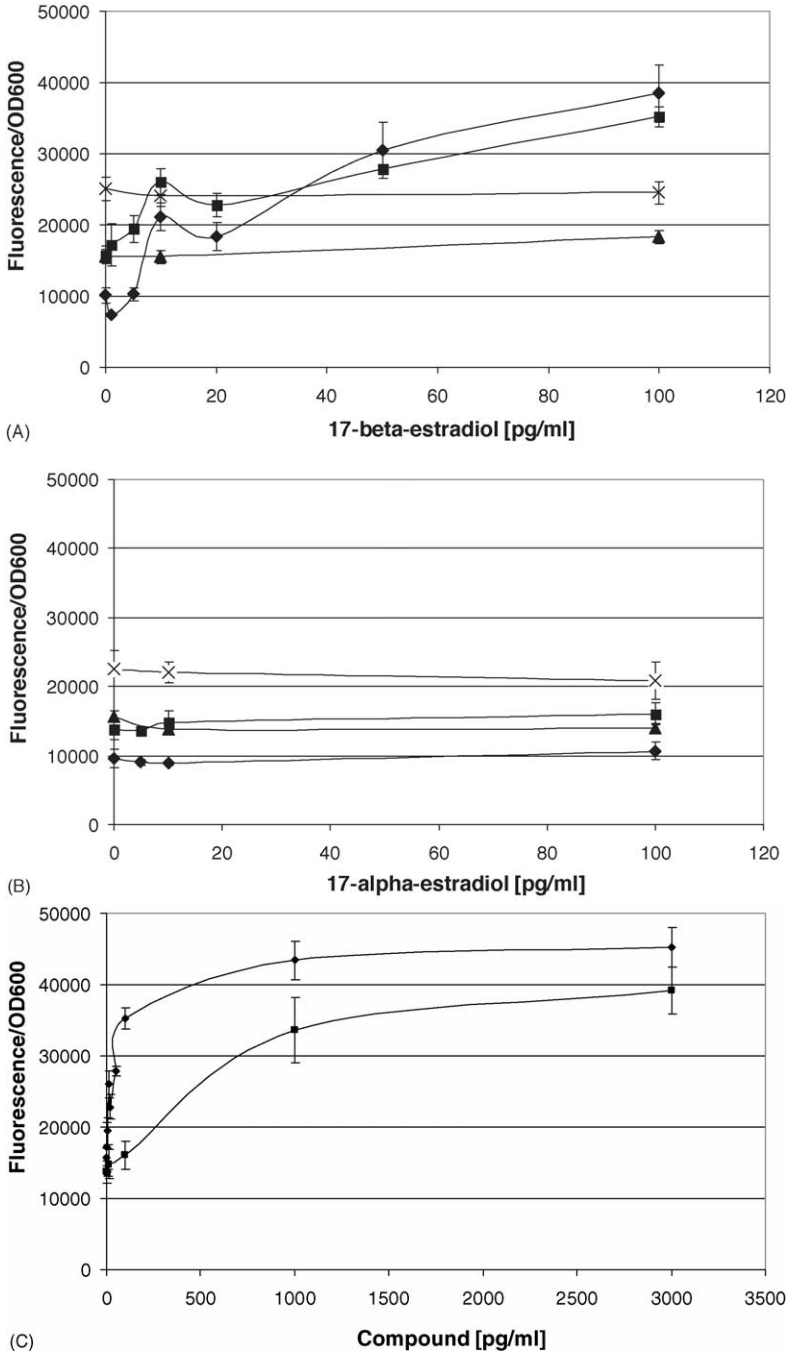

Fig. 1. Dose–response plots of *S. cerevisiae* strains after exposure to increasing concentrations of 17β-estradiol (A) or 17α-estradiol (B). Fluorescence emission was measured (excitation at 485 nm) at 535 nm, and the net development calculated by correction for blanks and negative controls and normalisation to the optical density (OD), representing growth observed over an incubation period of 16.5 h at 30 °C (starting OD 0.4/cm). Measurements were carried out with the *S. cerevisiae* wild type (♦) and the *pdr5 snq2 yor1* mutant (■) expressing the full-length human estrogen receptor and the GFP reporter plasmids or the C-terminal truncated, and thus non-functional human estrogen receptor and the GFP reporter plasmids (wild type (▲); FYAK (×)). The comparison of 17β-E2 (♦) and 17α-estradiol (■) in concentrations up to 3000 pg/ml is depicted in (C). Values represent the mean of quadruplicate samples of six (A), four (B) and five (C) independent experiments ±S.E.M.

emission and thus estrogenic activity of 17β-E2 decreased in *S. cerevisiae* wild type by ∼50% compared to that detected in DMSO solvent. In the *S. cerevisiae pdr5 snq2 yor1* mutant indicator strain, the estrogenic activity of 17β-E2 in charcoal-stripped human serum reached almost the same level of that in solvent with an identical lower detection limit of 5 pg/ml.

In order to test physiological conditions, complex human serum samples with previously defined concentrations between <5 and 50 pg/ml 17β-E2 by receptor immune de-

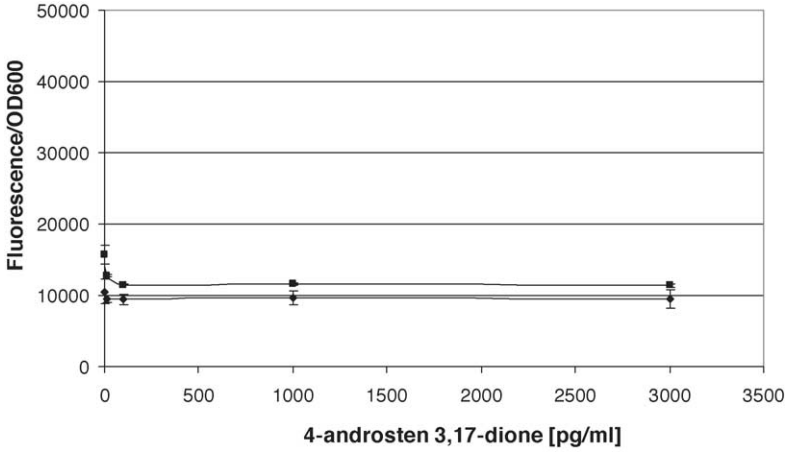

Fig. 2. Dose–response plot of *S. cerevisiae* strains after exposure to increasing concentrations of the androgen 4-androsten 3,17 dione. Fluorescence development was measured for 16.5 h as described in Section 2. Measurements were carried out with the *S. cerevisiae* wild type (♦) and the *pdr5 snq2 yor1* mutant (■) expressing the full-length human estrogen receptor and the GFP reporter plasmid. Values represent the mean of quadruplicate samples of three independent experiments ±S.E.M.

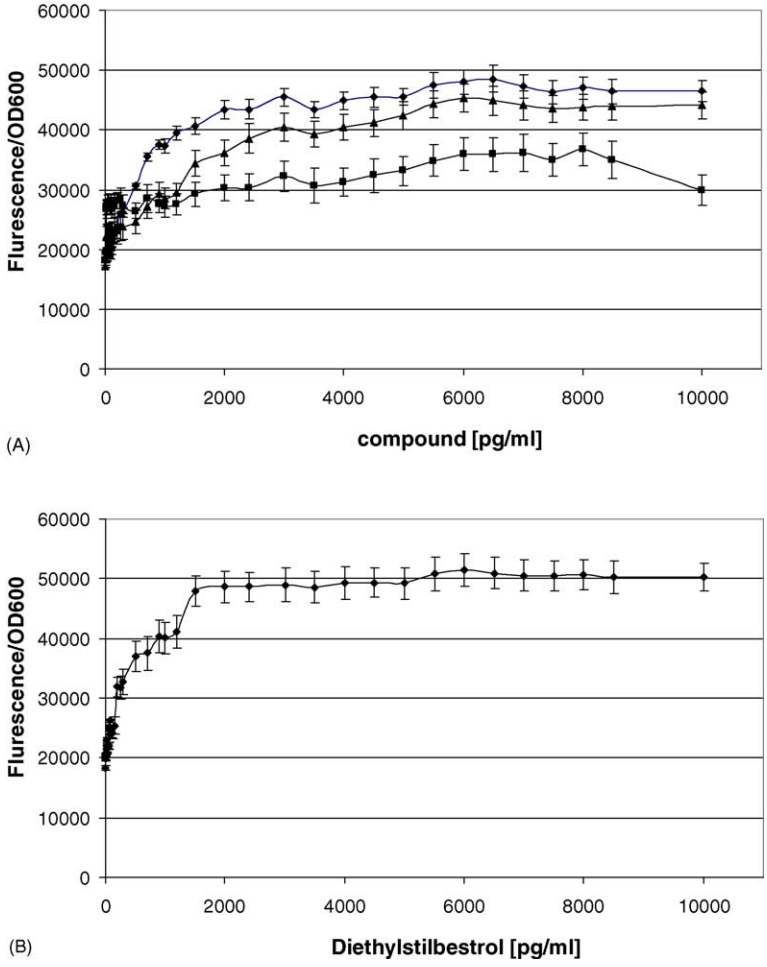

Fig. 3. (A) Dose response plot of *S. cerevisiae* strains after exposure to increasing concentrations of the phytoestrogens Coumestrol (♦), Zearalenon (▲) and Genistin (■). Measurements were carried out with the *S. cerevisiae pdr5 snq2 yor1* mutant expressing the full-length estrogen receptor together with the GFP reporter plasmid. Values represent the mean of quadruplicate samples of five independent experiments ±S.E.M. (B) Results for the synthetic estrogen, Diethylstilbestrol (♦). Values represent the mean of quadruplicate samples of five independent experiments ±S.E.M.

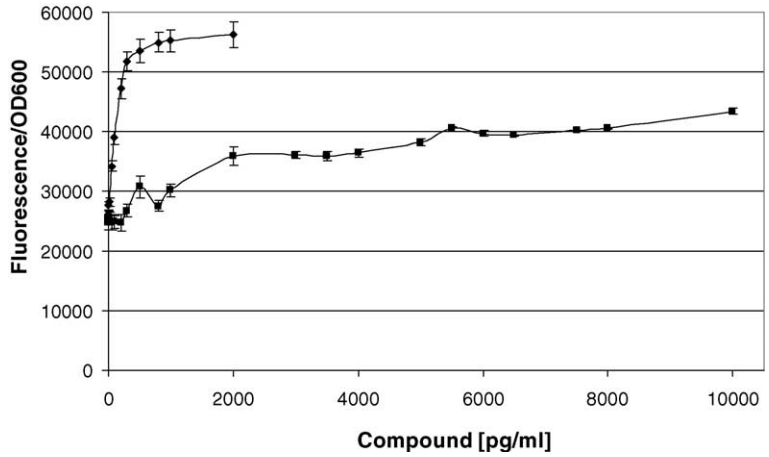

Fig. 4. Dose–response plot of *S. cerevisiae* strains after exposure to increasing concentrations of the estrogens ethinyl-estradiol (♦) and estradiol-3-benzoate (■). Measurements were carried out with the *S. cerevisiae pdr5 snq2 yor1* mutant expressing the full-length estrogen receptor together with the GFP reporter plasmid. Each value represents the mean of quadruplicate samples of four independent experiments with error bars giving the S.E.M.

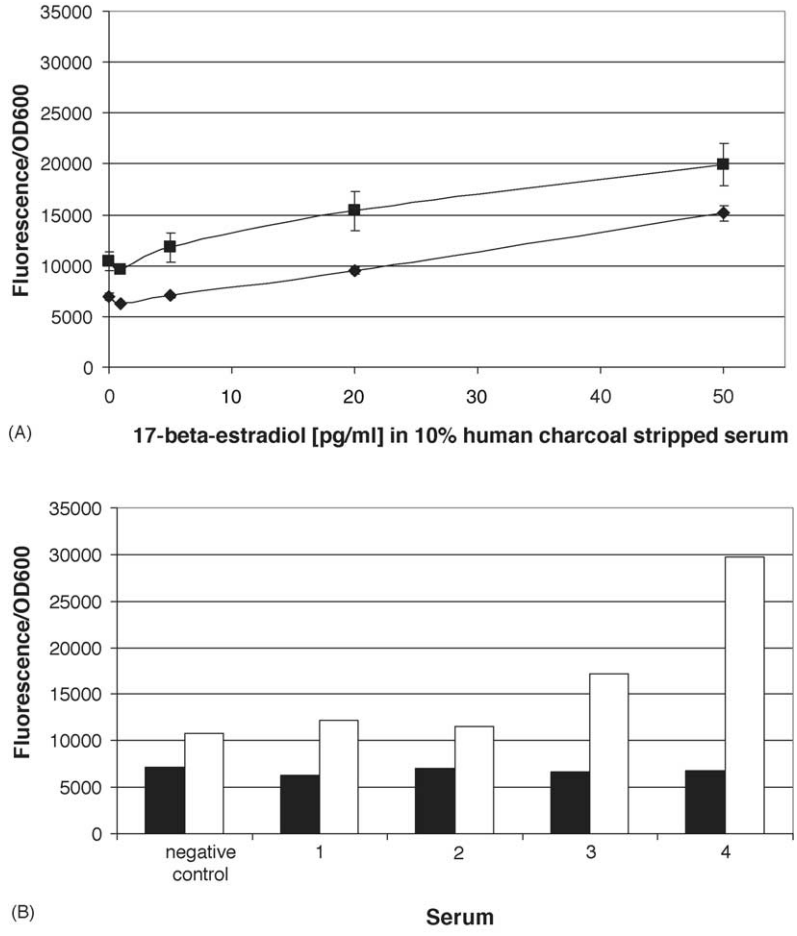

Fig. 5. (A) Calibration curve of increasing concentrations of 17β-estradiol in 10% human charcoal-stripped serum. Fluorescence development was measured for 16.5 h. Measurements were carried out with the *S. cerevisiae* wild type (♦) and the *pdr5 snq2 yor1* mutant (■) expressing the full-length human estrogen receptor and the GFP reporter plasmid. Each value represents the mean of quadruplicate samples of three independent experiments. (B) Detection of estrogenic bioactivity in native human serum samples. Corresponding concentration in the test culture were: negative control (10% serum), 0 pg/ml; serum 1, 0.2 pg/ml; serum 2, 5.1 pg/ml; serum 3, 13.1 pg/ml and serum 4, 54.8 pg/ml. The empty columns represent the *S. cerevisiae pdr5 snq2 yor1* mutant strain expressing the full-length receptor and the black columns represent the strain expressing the truncated receptor.

termination were examined with the *S. cerevisiae* strains (Fig. 5B). Corresponding concentrations were: negative control, 0 pg/ml; serum 1, 0.2 pg/ml; serum 2, 5.1 pg/ml; serum 3, 13.1 pg/ml and serum 4, 54.8 pg/ml. Estrogenic bioactivity could be observed in the *S. cerevisiae pdr5 snq2 yor1* mutant indicator strain in a concentration-dependent manner in contrast to the wild type.

4. Discussion

We investigated *S. cerevisiae* strains transformed with αhER and pERE-CYC1-GFP plasmids to identify and characterise estrogenic activity through the binding of the human estrogen receptor. Since yeast do not contain endogenous sex steroid receptors, the indicator strains expressing the full functional hER enable quantification of both the DNA binding and transcriptional activation function of the hER because the receptor is estrogen-induced and binds its own response element. Investigations comprised both sensitivity and specificity of the system.

4.1. Sensitivity

The sensitivity of the yeast GFP system was tested by serial dilutions of 17β-E2 with kinetic measurements of specific fluorescence development in a *S. cerevisiae* wild type and a *pdr5 snq2 yor1* mutant. Since the constructed non-functional receptor control strain did not yield any reporter response, upon 17β-E2-exposure, (Fig. 1A) in both genetic backgrounds, this control was only applied at random for all further experiments (data not shown). In yeast the presence of transporters homologous to P-glycoprotein has been identified. Mahe et al. [14] reported the export of estradiol by the transporter Pdr5. Also, the ABC-cassette transporter Snq2 plays a role in regulating the concentration of various chemicals [12]. Tran et al. [9] evaluated the effect of the ABC-cassette transporters on the activity of clinical and environmental anti-estrogens. In that study, 4-hydroxy tamoxifen only functioned as an anti-estrogen in the absence of the transporter Pdr5. The activity of other anti-estrogens was dependent on the presence or absence of specific transport proteins in yeast. Also, Banerjee et al. [18] recently reported the upregulation of the PDR5 gene upon steroid exposure. In the current test, the presence of the pleiotropic resistance-mediating transporters Pdr5, Snq2 and Yor1 did influence 17β-E2-induced GFP transactivation in both strains when applied in DMSO (Fig. 1A). The detection limit was 5 pg/ml (0.018 nM) in the *S. cerevisiae pdr5 snq2 yor1* mutant indicator strain in DMSO solvent compared to the wild type with a saturation limit at about 1000 pg/ml, which is in good agreement with other reported yeast [4,19,20,21] and MCF7-ERE [22] data and less than the detection limit of 0.1 nM reported by Bovee et al. [8] for a related bioassay. The presence of putative 17β-E2-binding proteins in 10% charcoal-stripped serum, which potentially could reduce the availability of the free agonist, did not generally affect the yeast hER-mediated transcriptional response. However, a clear difference between the two yeast strains was observed in 10% human charcoal-stripped serum (Fig. 5A). Under these conditions, the estrogenic activity of 17β-E2 in the *S. cerevisiae pdr5 snq2 yor1* mutant indicator strain was almost identical to that in solvent with an identical lower detection limit of 5 pg/ml, whereas the fluorescence emission and thus hER-dependent GFP transcription decreased in *S. cerevisiae* wild type by ∼50% compared to that in solvent. The latter observation is in agreement to what was earlier reported by Arnold et al. [19] who observed a decreased estradiol-mediated transactivation upon human serum incubation exposure in a *S. cerevisiae* wild type strain. It may be that the presence of a multitude of components in the human serum induces upregulation of the PDR transporters in competent wild type cells, and thus enhanced export of also 17β-E2. This effect was even more visible in the presence of native human serum samples, providing a realistic re-creation of physiological conditions (Fig. 5B). The mutant indicator strain responded appropriately to the exposure of native human serum that potentially contained additional estrogenic compounds other than 17β-E2, whose concentrations were estimated with the receptor immune determination.

17β-E2 is mainly produced in the ovaries and the plasma level of adult, pre-menopausal women is between 20 and 60 pg/ml, but can rise to levels of up to 400 pg/ml during the preovulatory E2-rise. In postmenopausal women, as in the human male, concentrations below 30 pg/ml are reported [23,24]. The yeast GFP test system using *S. cerevisiae pdr5 snq2 yor1* mutant cells may thus provide an advantageous sensitive assay for quantifying estrogenic bioactivity among physiological estrogen concentrations in the range between 5 and 1000 pg/ml when compared to standard curves.

4.2. Specificity

The specificity of the yeast GFP system was investigated with two approaches, the usage of a truncated version of the hER, lacking most of the C-terminal ligand-binding E domain and the application of a stereo isomer, 17α-estradiol and an androgen. The non-functional hER (YEpE12t608) did not mediate any response to increasing concentrations of 17α- and 17β-estradiol (Fig. 1) and thus served as a reliable technical control. The androgen 4-androsten 3,17 dione did not induce fluorescence development in cells expressing the functional hER (Fig. 2), even at 3000 pg/ml. The specificity of this system was also tested by the application of the phytoestrogens: Coumestrol, Genistin, the mycotoxin Zearalenon (Fig. 3A), the synthetic estrogen DES (Fig. 3B) and the estrogenic compounds ethinyl-estradiol and estradiol-3-benzoate (Fig. 4). All of these yielded a substantially lower biological estrogenic activity as the true agonist 17β-E2. For DES, which is known to be as potent as 17β-E2 in other in vitro assays, the significantly reduced activity in this test might be due to the fact that DES inhibits the yeast plasma membrane

ATPase Pma1, thus depleting the available metabolic energy. The ester estradiol-3-benzoate does not bind to hER but requires hydrolysis of the ester bond prior to the expression of estrogenic activity. The very weak, but unsaturable activity of this compound in our test might be due to the unspecific activity of a variety of yeast esterases, e.g. Tgl1, which is assumed as steryl ester hydrolase [25], and was also described to enhance steroid productivity [26].

Interestingly, the estrogen epimer 17α-estradiol (Fig. 1B) did not induce a true hER-dependent agonist response in cells expressing the functional receptor (YEpE12). Only at approximately 1500 pg/ml, an estrogenic bioactivity by similar fluorescence emission compared to 100 pg/ml 17β-E2 was observed.17α-E2 is different from 17β-E2 in the steric position of the hydroxy-group at the 17 C-atom and the major estrogen in ungulates and rodents [1]. In humans, 17α-estradiol has only been found under some specific (rare) circumstances in the serum and urine [2,27], it is generally considered as being devoid of classical biological estrogen activity. However, 17α-E2 and some sulfate derivatives are components of complex estrogens compounds applied in the treatment of peri- and postmenopausal women. According to a clinical study, monomeric 17α-E2 is equally capable as 17β-E2 to alleviate menopausal hot flushes [28]. By using radio receptor-assays, a much weaker affinity of 17α-E2 to the hER compared to 17β-E2 was shown in that study, which is in good agreement to the current results obtained with the yeast-based test system. This may provide evidence that 17α-E2 could be an alternative substance for the treatment of menopausal symptoms, whose effect is not hER-dependent but mediated by other mechanisms, for instance the inhibition of the catechol-*O*-methyltransferase. In view of the current controversial discussion of the usual 17β-E2 substitution therapy of postmenopausal symptoms (and suspected tumor promoting activity), additional in vitro and in vivo characterisation of 17α-E2 bioactivity could be worthwhile.

The human estrogen receptor, a member of the superfamily of ligand-activated transcription factors, mediates the effects of estrogens on sexual development, reproduction and growth [29]. The binding of an estrogen to hER induces a cascade of events, including the release of accessory proteins (e.g. the heat-shock proteins), increased nuclear retention, DNA binding and the transcription of estrogen-responsive genes [30]. To avoid the complexity with mammalian systems and in view to both, understanding the molecular mechanisms underlying hormone-dependent activation and assessment of a large number of natural and xeno-estrogens a variety of in vitro [31] including yeast (anti-) estrogenic screens have been developed [3–6,8]. Many of these employ the transcriptional activation of β-galactosidase, and upon cell lysis, the measurement of the dye reaction. The green fluorescence protein production of hER-transformed *S. cerevisiae* cells in relation to controls with and without 17β-estradiol (E2) was successfully established as a simple screening system for the quantitative determination of estrogenic bioactivity of steroids and native samples. With a detection limit for E2 of 5 pg/ml (0.018 nM) in the *S. cerevisiae pdr5 snq2 yor1* mutant indicator strain, even in the presence of native human serum and a capability to distinguish between agonists, this screening system offers a sensitive, fast and reliable method for potential routine testing of estrogen bioactivity in biological samples, including those obtained from women before or during hormone replacement therapy.

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