Archives of Biochemistry and Biophysics 541 (2014) 61–66

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Impact of biochemical design on estrogen receptor/estrogen response element interaction by surface plasmon resonance technology

Denis Habauzit [a,b,*], Sandrine Bayle [a], David Benimelis [c], Joel Chopineau [c,d], Benoit Roig [c,e,f]

[a] Laboratoire Génie de l'Environnement Industriel, Ecole des Mines d'Alès, 6 Avenue de Clavières, 30319 Alès Cedex, France
[b] INSERM U1085, Institut de Recherche en Santé, Environnement et Travail (IRSET), Equipe "TREC", Université de Rennes 1, SFR Biosit, Campus de Beaulieu, Bat 13, 263 avenue du Général Leclerc, CS 74205, 35042 RENNES cedex, France
[c] Université de Nîmes, Rue Georges Salan, 30021 Nîmes, France
[d] Institut Charles Gerhardt Montpellier, UMR 5253 CNRS-ENSCM UM2-UM1, Ecole Nationale Supérieure de Chimie, Montpellier, France
[e] EHESP Rennes, Sorbonne Paris Cité, Avenue du Professeur Léon Bernard, CS 74312, 35043 Rennes Cedex, France
[f] INSERM U1085-IRSET, LERES, France

ARTICLE INFO

Article history:
Received 13 September 2013
and in revised form 23 October 2013
Available online 23 November 2013

Keywords:
Surface plasmon resonance
Estrogen
Estrogen receptor
Dimerization
Solvent
Temperature

ABSTRACT

The estrogen receptor (ER) is a transcription factor that binds under 17-β-estradiol (E2) stimulation as homodimer to a short DNA consensus sequence named estrogen response element (ERE). The ER/ERE interaction has been assessed by several research groups through different methodologies notably by surface plasmon resonance (SPR) techniques. The biochemical parameters and conditions (solvent, ER concentration, salt, time and temperature) used to prepare samples before analysis were very different from one study to another. But no studies have aimed to compare the effect of these modifications on ER/ERE interaction. Therefore the main objective of the present paper was to assess the influence of biochemical parameters onto the ER/ERE interaction with the final aim to improve the comprehension of this interaction. Our results highlighted that parameters like solvent, ER concentration, salt and surfactant concentration, temperature and time deeply modify ER/ERE interaction. Nevertheless, the dimer formation under E2 stimulation occurred with all tested conditions. Altogether, incubation parameters of ER with E2, deeply modify its binding level onto ERE. These data constitute an important key point to consider for the improvement of ER/ERE detection method depending upon the aim of the study (interaction measurement, environmental detection, development of new technologies or devices).

© 2013 Elsevier Inc. All rights reserved.

Introduction

The estrogen receptor (ER)[1] belongs to the family of nuclear receptor [1]. In cell the activity of this transcription factor (TF) is mainly due to its activation by steroid especially 17-β-estradiol (E2). In cells, this TF is implicated in several cellular function and orientation like in the proliferation [2], in the differentiation [3,4], in the protection [5], and finally in gene expression.

Several experiments have been developed for the comprehension and for the characterization of the mechanism by which ER is activated by ligand and its binding capacity as a homodimer $(ER/E2)_2$ to a specific DNA consensus sequence (ERE) located upstream of some estrogenic regulated genes. In this way several biophysical tools have been developed to explore the interaction phenomena such as fluorescence anisotropy [6–8], surface plasmon resonance (SPR) [9–12], FRET [13], electrochemical [14–16], resonant waveguide [17]. The main objectives were to determine the estrogenic compounds/ER interaction properties [18–20], the ER/ER dimerization phenomena [13,21], ER/ERE interaction mechanisms and properties under estrogenic stimulation [9,10].

Now these interactions are used for several purposes such as (i) comprehension of molecular interaction; (ii) measurement of ER interaction properties; (iii) evaluation of the estrogenic potential of compounds that mimic the natural hormone; (iv) the development of new methods for environmental monitoring with direct and fast quantification of estrogenic compounds and finally; (v) their use as a classical model of interaction for the development of new devices or apparatus for the biophysical measurement.

* Corresponding author at: Institut de Recherche en Santé, Environnement et Travail (IRSET), Inserm-U1085, Equipe "Transcription, Environnement et Cancer", Université de Rennes 1, SFR Biosit, Campus de Beaulieu, Bat 13, 263 avenue du Général Leclerc, CS 74205, 35042 RENNES cedex, France. Tel: +33 2 23 23 61 32.
E-mail addresses: denis.habauzit@univ-rennes1.fr, benoit.roig@unimes.fr (D. Habauzit).

[1] *Abbreviations used:* ER, estrogen receptor; ERE, estrogen response element; E2, 17-β-estradiol; SPR, surface plasmon resonance; RU, resonance unit; TNMT, Tris Nacl MgCl tween buffer; SCSA, sensor chip surface streptavidin's coated; DMSO, dimethyl sulfoxide; Na, not available.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.11.006

Among biophysical methods, SPR allows real time monitoring of direct interaction between molecules. The signal variation, expressed in resonance unit (RU), is proportional to the amount of bound molecules [11]. Recently, SPR technology has been extensively developed in the environmental field, especially for the assessment of molecular interactions or for the detection of pollutant [11,22]. These developments are first due to its sensitivity without a pre-concentration step (low detection limit (ng/L)) [23]. Then, there is currently a high development of miniaturized portable systems [24]. However this technique requires knowledge on the interaction considered: in the case of estrogenic compounds, the interaction between the compound (like E2) and the estrogen receptor (ER) characterized by ER dimerization and bounding with the nucleic acid (ERE). Different SPR systems have been used to characterise notably the estrogenic compounds/ER interaction properties [18–20], the ER/ER dimerization [21], the ER/ERE binding mechanisms [9,10,12,25–31], and ER interaction with some other transcription factors like SP1 [32].

In the characterization of (ER/E2)/ERE interaction [9,10,12,25–31] (Fig. 1), various experimental conditions have been used such as solvent to dissolve E2, the range of ER concentration and the ER/E2 incubation parameters (Table 1) without any justifications. The diversity of the experimental conditions makes the establishment of this experimental approach difficult. Moreover, these variations of experimental design could explain few authors did not reproduce the increase of the ER binding level by SPR in the presence of E2 [30]. Our work aims to homogenate protocol depending onto the aim of the study. Therefore we evaluate the impact of (i) the solvent used in ER/ERE and on (ER/E2)/ERE interactions; (ii) the ER concentrations (10 or 50 nM); (iii) the buffer salt concentration; (iv) the temperature and time effects on dimer formation; and (v) the temperature and time effects onto the dimer preservation. We aim to determine the optimum conditions for (ER/E2)/ERE interaction analysis by considering the dimer formation best conditions, its preservation and its detection *via* a SPR system either for its use in fundamental studies or in screening purpose.

Materials and methods

Reagents

All chemicals used in this work were of analytical grade. Tris buffer, Tween 20, dimethyl sulfoxide (DMSO), methanol and 17-β-estradiol (E2) were purchased from Sigma–Aldrich (St Quentin Fallavier, France).

TNMT (Tris, NaCl, $MgCl_2$, and Tween) buffer contains 50 mM Tris HCl, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% Tween 20, pH 7.5. Three additional modified TNMT buffers were prepared: TNMT methanol (0.2% vol/vol), TNMT DMSO (0.2% vol/vol) and TNMT solvent free buffers. In the last one, E2 is prepared firstly in methanol then 2 µL is evaporated and E2 (solvent free) is dissolved in TNMT.

Human recombinant ERα and Estrogen Response Element (ERE) used have been previously described [10].

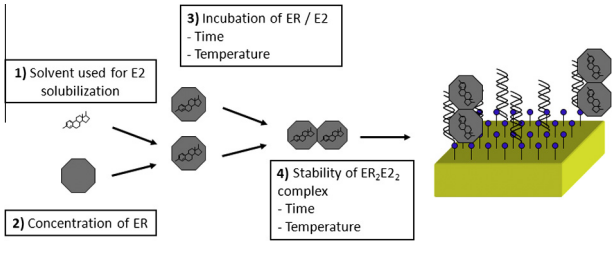

Fig. 1. Assay design used in this study.

Solutions preparation

E2 solution

E2 was dissolved in methanol or in DMSO and added to running buffer to prepare E2 standards with final concentrations ranging from 2 to 2000 nM. Final methanol or DMSO concentrations were 0.2% (vol/vol) in TNMT buffer or milliQ water.

ER solution

ER solutions were diluted in TNMT buffer for final concentration of 20, 30 or 100 nM.

ER/E2 solution

A volume of buffer containing or not E2 was mixed with an equal volume of ER solution. The final concentration of solvent and of ER was therefore 0.1% and 10, 15 or 50 nM, respectively.

SPR experiments

Biacore analysis has been performed at 25 °C on Biacore 1000 apparatus. ERE was firstly bound onto the activated sensor chip surface streptavidin's coated (SCSA) by injection of ERE solution (450 nM diluted in running buffer) at a flow rate of 5 µL/min.

The ER/ERE interaction was measured by injecting 40 µL of the ER in the presence or in the absence of E2 onto the ERE bound sensor chip surface with a constant flow rate of 20 µL/min. After reversion to running buffer, the dissociation phase was recorded for 180 s. After each injection, the surface was regenerated by injection of 20 µL of a 0.1% SDS solution (20 µL/min) followed by rinsing with running buffer for 2 min. One cycle of regeneration was enough to remove all bound proteins.

The quantification of the binding ER/ERE was determined from the sensorgram 10 s after the reversion to running buffer.

Incubation kinetics for the binding of ER with E2 and the binding level offer onto ERE were assessed at different temperatures 0.1, 21.6, 25, 26.5, 30 °C and compared to the reference incubation conditions (overnight at 4 °C) determined in previous works [10]. Some experimental parameters such as conditioning of the surface and running buffer alone (TNMT buffer) have been previously described [10].

Results and discussion

Fig. 1 and Table 1 summarize the method and the experimental parameters generally described in the literature for the detection of estrogenic compound by SPR (based on ER/ERE interaction). However, the parameters differ from one study to another as for example:

- The solvent for E2 solubilization was either DMSO, ethanol or methanol and was used interchangeably without any justification.
- The concentrations of ER ranged from 10 up to 270 nM. These concentration values could influence the concentration of E2 needed to induce the ER dimerization.
- The conditions of temperature and time used for ER/E2 incubation (to reach the dimerization of ER in the presence of E2) range from 37 °C/5 min to 4 °C/overnight.

However, for a more widespread use of SPR in this purpose, there is a need of standardized protocols. In the following, we tried to bring a light on the best conditions needed for the ER/ERE interaction study by SPR either for fundamental study purpose as well as for optimizing our endocrine disrupting chemical detection method. 17-β-Estradiol (E2) was chosen as model estrogenic compounds.

Download English Version:

https://daneshyari.com/en/article/1925286

Download Persian Version:

https://daneshyari.com/article/1925286

Daneshyari.com