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Interaction of ERp57 with calreticulin: Analysis of complex formation and effects of vancomycin

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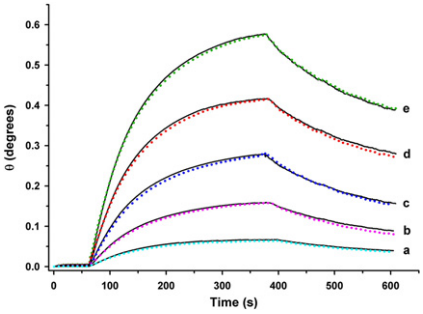
HIGHLIGHTS

- SPR data provide evidence for an ERp57 conformational change in the binding to CRT.
- Vancomycin could hinder the stabilizing conformational change in ERp57—CRT complex.
- Vancomycin decreases the amount of calreticulin on plasma membrane.

GRAPHICAL ABSTRACT

Sensorgrams for the affinity interaction of variable concentration of CRT to immobilized ERp57 in the presence of vancomycin. The concentrations of calreticulin (μM) were: 0.5 (a), 1.0 (b), 2.0 (c), 3.0 (d) and 4.0 (e).



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ABSTRACT

The protein ERp57 (also known as PDIA3) is a widely distributed protein, mainly localized in the endoplasmic reticulum, where it acts as disulfide isomerase, oxidoreductase and chaperone, in concert with the lectins calreticulin (CRT) and calnexin. The ERp57/CRT complex has been detected on the cell surface and previous studies have suggested its involvement in programmed cell death. Although the ERp57-CRT complex has been characterized, little is known about its role in different cellular compartments as well as inhibitors of this interaction.

We focused on the kinetic, extent and stability of the ERp57-CRT complex, using the surface plasmon resonance spectroscopy, investigating the possible role as inhibitor of the antibiotic vancomycin. Equilibrium thermodynamic data suggested that vancomycin may hinder the interaction between the two proteins and could interfere with the ERp57 conformational changes that stabilize the complex. Furthermore, by means of confocal microscopy, we evaluated the effect of the in vivo administration of vancomycin on the ERp57/CRT complex on the surface of HeLa cells.

The model presented here could be used for the search of other specific inhibitors/interactors of ERp57, which can be extremely helpful to understand the biological pathways where the protein is involved and to modulate its activity.

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1. Introduction

ERp57 (endoplasmic reticulum p57, EC 5.3.4.1, also known as PDIA3, ERp60 and GRP58) is a soluble protein, member of the disulfide isomerases family (PDIs), which is mainly located in the lumen of the endoplasmic reticulum (ER). Like the proteins belonging to the glucose-regulated proteins (GRP) family, ERp57 is overexpressed in response to glucose deprivation and to glycosylation blocking reagents [1], but it is also induced by many other cellular stress conditions, such as depletion of calcium stores from ER, presence of misfolded proteins, reductive stress, hypoxia, and low pH [2]. The location of ERp57 is not limited to the ER. In fact, it has been found in different subcellular compartments, including nucleus [3], cytoplasm [4] and cell surface membrane [5,6,7,8].

The role of ERp57 in the ER is relatively well understood. In this compartment, ERp57 acts as disulfide isomerase, oxidoreductase and chaperone, participating in the folding of newly synthesized glycoproteins, in concert with the lectins calreticulin (CRT) and calnexin [9].

In addition to the endoplasmic location, calreticulin has been localized in the cytoplasm [10], associated with the vitamin D receptor (VDR) together with other members of the steroid receptor protein superfamily [11] and involved in the nuclear export [12,13].

The function of the ERp57-CRT complex is well known in the ER, however the complex re-localization between different cellular compartments is still poorly understood. A recent research has revealed that calreticulin co-translocates to the cell surface in association with ERp57 [14]. It has been demonstrated that the exposure of calreticulin on the plasma membrane precedes anthracycline-induced apoptosis and that it is required for cell death to be perceived as immunogenic [15].

To date, a fully crystallographic structure of ERp57 is limited to a covalent complex with tapasin [16]. In the same report, the Authors suggested an in silico model for the luminal subcomplex of the peptide-loading complex, made up with tapasin, ERp57, MHC class I, and calreticulin. The ERp57-CRT complex has been characterized and it was demonstrated that the P domain of calreticulin binds the b and b′ domains of ERp57 [17, 18]. Moreover, Kimura et al. [19] have analyzed the ERp57-CRT interaction and demonstrated that ERp57 competitively forms complexes with PDI and CRT.

In this paper we focused the attention on the specific interaction between ERp57 and calreticulin. The kinetic and affinity of the binding of recombinant ERp57 to calreticulin has been investigated by surface plasmon resonance (SPR) spectroscopy. The SPR technique has recently demonstrated to be an interesting tool in biomolecular interactions, providing kinetic information along with affinity data which can be employed for thermodynamic studies [20, 21]. The extent of complex formation as a function of the temperature has been also evaluated, enabling the calculation of both equilibrium and transition-state thermodynamic parameters for this fundamental molecular recognition process. Considering that the physiological role and functions of ERp57 within the cell are not fully elucidated, the identification of specific inhibitors capable to perturb the ERp57 interactions, such as the ERp57-CRT complex formation, is highly desirable. It has been previously reported that ERp57 interacts with some antibiotics which interfere with its activity. In particular, vancomycin has been shown to bind reversibly to ERp57 and to inhibit the reductase and the DNA-binding activities of the protein [22]. In this context, the possible role of vancomycin as inhibitor of the ERp57-CRT interaction and its effect on the stability of the protein complex have been also investigated.

The experimental model presented here can be exploited for the detection of other specific inhibitors of ERp57, which could be used with greater effectiveness in vivo.

2. Materials and methods — experimental procedures

2.1. Materials

Human recombinant ERp57 was obtained as previously described [23]. Mouse monoclonal anti-ERp57 (sc 23886) and rabbit polyclonal anti-calreticulin antibodies were from Santa Cruz. Vancomycin and calreticulin were from Sigma.

2.2. SPR binding experiments

SPR experiments were performed with Eco Chemie Autolab SPR system (Ecochemie, The Netherlands). ERp57 protein was covalently coupled to a CMD20 SPR sensor chip (Xantec Bioanalytics GmbH, Munster, Germany) through its free amino groups using a mixture containing 0.5 mM ethyl(dimethylaminopropylcarbodiimide) (EDC) and 0.1 mM N-hydroxysuccinimide (NHS). To prepare a high-capacity surface, a 50 μl aliquot of ERp57 solution (0.1 mg/ml) was used.

Measurements of rate and extent of interaction between the immobilized protein and calreticulin over a concentration range were performed by monitoring the changes in the resonance angle shift at the SPR surface. Signals were collected at temperatures ranging from 10 to 35 °C and in the presence of 10 mM Tris–HCl, pH 8.0, as coupling buffer. The data were corrected for changes in the refractive index because of solvent mismatches, injection noise as well as non-specific peptide binding to the ligand-free surface.

For the study of interaction between ERp57 and vancomycin, the latter was covalently immobilized on a mixed self-assembled monolayer (SAM) on gold generated from the (2-(2-(2-(11-mercaptoundecyl-oxy)-ethoxy) ethoxy) ethyl alcohol, $(HS(CH_2)_{11}(OCH_2CH_2)_3OH)$, **1**, and the (2-(2-(2-(2-(2-(2-(2-(11-mercaptoundecyl-oxy)ethoxy)ethoxy)ethoxy)ethoxy) ethoxy) ethoxy) acetic acid, $(HS(CH_2)_{11}\text{-}(OCH_2CH_2)_6OCH_2CO_2H)$, **2** (Scheme S1, Supplementary data). A solution 1 mM of vancomycin was added on the modified surface, after pre-activation of the carboxylic groups with a mixture containing 0.5 mM EDC and 0.1 mM NHS in PBS for 30 min. The modified surface was incubated for 1 h with insulin at 4 °C. After being rinsed with deionized water, the substrates were blown dry under N_2 and used for the SPR measurements. For more details about the synthesis of compounds **1** and **2**, and surface modification see Supplementary data.

2.3. Cell culture

Human cervical adenocarcinoma (HeLa) cells were grown at 37 °C and 5% CO_2 in DMEM medium, supplemented with 10% (v/v) fetal bovine serum, 1% sodium pyruvate, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin.

2.4. Immunofluorescence analysis

HeLa cells were grown on glass coverslips and treated with 100 nM, 500 nM and 1 μM vancomycin for 10 min at 37 °C or left untreated as a control. Cells were washed with serum-free medium and PBS, then fixed in 4% paraformaldehyde for 20 min. In parallel, additional untreated control cells (C*) and 500 nM vancomycin treated cells (500*) were permeabilized with 0.1% Triton X-100 in PBS. Both non-permeabilized and permeabilized cells were then washed with 0.5% BSA in PBS, and incubated for 1 h in 1% BSA in PBS (PBS/BSA). To detect calreticulin, cells were incubated for 1 h with anti-calreticulin (rabbit polyclonal antibody) (1:30 dilution in PBS/BSA), washed in PBS/BSA, and incubated for 1 h with TRITC-conjugated goat-anti-rabbit-IgG (Jackson Immunoresearch) in the dark. To detect ERp57, cells were washed and stained with anti-ERp57 (mouse monoclonal antibody) (1:30 dilution) followed by washing and incubation for 1 h with FITC-conjugated anti-mouse-