Available online at www.sciencedirect.com
ScienceDirect
Analytical Biochemistry 374 (2008) 133–142
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Interactions of fusidic acid and elongation factor G with lipid membranes

Jaana Muhonen [a], Jukka Vidgren [a], Anne Helle [a], Gebrenegus Yohannes [a], Tapani Viitala [b], Juha M. Holopainen [c,*], Susanne K. Wiedmer [a,*]

[a] *Laboratory of Analytical Chemistry, Department of Chemistry, 00014 University of Helsinki, Finland*
[b] *KSV Instruments, 00380 Helsinki, Finland*
[c] *Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, 00290 Helsinki, Finland*

Received 28 August 2007
Available online 14 October 2007

Abstract

Fusidic acid (FA) is a potent antibiotic and blocks the protein synthesis by binding to elongation factor G (EF-G) directly. Here we hypothesized that the antibiotic activity of FA would be potentiated by several orders of magnitude if both FA and EF-G would be residing in the lipid membranes and, hence, the probability of interaction would transform from three-dimensional to two-dimensional. Such detailed information could lead to more effective therapeutic interventions if they are understood on a molecular level. Interactions between FA and various lipid membranes composed of 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) and cholesterol (Chol) were studied by capillary electrochromatography (CEC). The influence of the lipid vesicle size—sonicated liposomes and liposomes extruded through 30-, 50-, and 100-nm filters—on the packing of vesicles on the silica capillary surface was investigated by CEC and dissipative quartz crystal microbalance. The CEC results evidenced that FA interacts with and resides in phospholipid membranes. Likewise, monolayer, asymmetrical flow field flow fractionation, and CEC studies confirmed that EF-G is hydrophobic and incorporated into POPC and POPC/Chol membranes. Including EF-G in phospholipid vesicles did not improve the binding of FA to the membranes.

Keywords: Capillary electromigration techniques; Elongation factor G; Field flow fractionation; Fusidic acid; Liposomes; Quartz crystal microbalance

Fusidic acid (FA)[1] is a hydrophobic, steroid-based, narrow spectrum antibiotic derived from *Fusidium coccineum.* It is a weak acid (pK_a of 5.7) and is mostly ionized in plasma and tissue at the physiological pH of 7.4 [1]. FA is used mainly to treat bacterial infections caused by gram-positive (G^+) bacteria such as *Staphylococcus aureus.* Yet it is also effective against gram-negative (G^-) *Neisseria* species as well as corynebacteria, nocardia, and anaerobes [2].

The synthesis of new proteins in living cells occurs on the ribosome. The elongation factor G (EF-G) plays an important role in the elongation phase of the protein synthesis by catalyzing the translocation of the peptidyl transfer RNA (tRNA) from the A-site to the P-site of the ribosome. EF-G is bound to the ribosome in a complex with guanosine triphosphate (GTP). After hydrolysis of GTP to guanosine 5′-diphosphate (GDP) and followed by translocation, EF-G–GDP is dissociated from the ribosome. The crystal structure of EF-G has been solved [3,4].

* Corresponding authors. Fax: +358 9 191 50253.
E-mail addresses: juha.holopainen@hus.fi (J.M. Holopainen), susanne.wiedmer@helsinki.fi (S.K. Wiedmer).

[1] *Abbreviations used:* FA, fusidic acid; EF-G, elongation factor G; tRNA, transfer RNA; GTP, guanosine triphosphate; GDP, guanosine 5′-diphosphate; CEC, capillary electrochromatography; POPC, 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine; Chol, cholesterol; QCM, quartz crystal microbalance; AsFlFFF, asymmetrical flow field flow fractionation; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GMP–PNP, guanosine 5′-(β-γ-imido)triphosphate; BGE, liposome solvent and electrolyte; EOF, electroosmotic flow; LUV, large unilamellar vesicle; HPLC, high-performance liquid chromatography; CZE, capillary zone electrophoresis; QCM-Z, quartz crystal microbalance based on impedance analysis; 2D, two-dimensional; 3D, three-dimensional.

0003-2697/$ - see front matter
doi:10.1016/j.ab.2007.10.014

Cryo-transmission electron microscopy studies have shown that the binding of EF-G–GTP to the ribosome and the following hydrolysis of GTP causes large structural changes in the ribosome and in EF-G [5,6]. FA blocks protein synthesis by inhibiting EF-G directly [7]. FA binds with high affinity to the EF-G-GDP after the hydrolysis of GTP and prevents the release of EF-G–GDP complex from the ribosome [8], thereby stalling protein synthesis. There is no structural evidence for the existence of an EF-G–GDP–FA complex without the presence of ribosomes. Yet indirect evidence for FA–EF-G complex has been presented [9].

For FA to reach intracellular EF-G, it first must interact with bacterial membranes. Then it may either randomly diffuse within the bacteria to an EF-G-containing location or remain partitioned within the membrane. In the latter case, EF-G must be attached to the bacterial membranes, where it may interact with FA. Ribosomes are able to interact directly with lipid membranes [10]. Accordingly, detailed knowledge of FA–lipid membrane and EF-G–membrane interactions is essential to understand, and possibly to enhance, the antimicrobial activity of FA and similar agents. The aim of this study is to fill this gap.

The hydrophobicity of compounds is linked directly to their membrane partitioning. The high capacity of FA for tissue penetration has been ascribed to the surface activity [11], and the lipid solubility of this drug has been studied only recently [12]. We have studied the partitioning of FA into lipid bilayers and FA–phospholipid interactions both experimentally—by means of capillary electrochromatography (CEC), differential scanning calorimetry, and fluorescence spectroscopy—and through molecular dynamics simulations [12]. We showed that FA partitioned readily into the lipid bilayer and possibly was enriched in lipid rafts. This may be followed by the association of EF-G–GDP with FA-enriched lipid domains.

Here we extend our previous study and further elucidate interactions between FA and various lipid membranes composed of 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) and cholesterol (Chol) by CEC. In addition, the influence of the lipid vesicle size on the packing of vesicles on the silica capillary surface was investigated by CEC and dissipative quartz crystal microbalance (QCM). The interaction of EF-G with lipid membranes was assessed by monolayer, asymmetrical flow field flow fractionation (AsFlFFF), and CEC studies. The presence of EF-G in the lipid membrane on the retention of FA was of specific interest. The goal was to acquire more information about the interactions of FA and/or EF-G with lipid membranes.

Materials and methods

Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), POPC, GDP, guanosine 5′-(β-γ-imido)triphosphate (GMP–PNP), atenolol, dichlorphenamide, and FA were purchased from Sigma Chemical (St. Louis, MO, USA). β-Cholesterol was obtained from Avanti Lipids (Alabaster, AL, USA). Testosterone, $MgCl_2$, and pH solutions (7 and 10) used for calibrating the pH meter were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1.0 M) and nitric acid (1.0 M) were obtained from FF Chemicals (Yli Ii, Finland), methanol was obtained from Mallinckrodt Baker (Deventer, The Netherlands), and chloroform was obtained from Rathburn Chemicals (Walkerburn, UK). Distilled water was further purified with a Millipore Water Purification System (Millipore, Molsheim, France). EF-G was expressed and purified as described previously and was kindly donated by Suparna Sanyal (Department of Cell and Molecular Biology, Uppsala University) [13].

Buffer and sample preparation

Hepes-containing buffer solution, with an ionic strength of 20 mM and pH 7.4 (adjusted with 1.0 M sodium hydroxide), was used as liposome solvent and electrolyte (BGE) solution. Before use, the BGE was filtered through a 0.45-μm syringe filter (Gelman Sciences, Ann Arbor, MI, USA). The samples for CEC studies were prepared from stock solutions of atenolol, aldosterone, testosterone, and dichlorphenamide (1.0–2.8 mg ml^{-1} in methanol). For CEC runs, the concentrations of the analytes in the injected sample were 40 μg ml^{-1} all in 5:95% (v/v) methanol/BGE solution. The concentration of FA, when used as an analyte, was 1 mM. The electroosmotic flow (EOF) in the capillaries was measured using 5 to 7.5% methanol in BGE solution as a neutral marker.

The stock solution of EF-G in water (400 μM) was diluted to 200 μM with 0.02% Na-azide and further diluted to 196, 167, 54, 30, 20, 10, 5, 2.5, or 1.25 μM into phospholipid solutions in BGE. The numbers of EF-G molecules per phospholipid molecule were then 1:0.1, 1:1, 1:12.5, 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800, respectively. The pure EF-G sample was diluted with BGE solution to 4.8 μM. The stock solutions of GDP and GMP–PNP were made in BGE in the concentration of 4 mM, and final concentrations in the coating solutions were 200 to 300 μM (and the final concentration for $MgCl_2$ was 5 mM). Phospholipid dispersions were extruded through a 50-nm membrane before the addition of EF-G, GDP, GMP–PNP, or $MgCl_2$.

POPC, Chol, and FA stock solutions in chloroform and EF-G, GDP, and GMP–PNP were stored in a freezer. All other solutions were stored in a refrigerator.

Liposome preparation

Liposome vesicles were prepared from POPC, Chol, and FA stock solutions (20 mM in chloroform). Liposomes were prepared as described previously [14]. Appropriate amounts of the lipid stock solutions in chloroform were mixed to obtain the desired compositions. The resulting