Micron 110 (2018) 73–78

Contents lists available at ScienceDirect

Micron

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

Impact of Cu(II)-doping on the vulnerability of *Escherichia coli* ATCC 10536 revealed by Atomic Force Microscopy

Wasia Rasheed[a,b,*], Samina Perveen[b], Ghulam Mustafa[b], Muhammad Raza Shah[b], Shakil Ahmed[b], Sami Uzzaman[b]

[a] Department of Applied Chemistry and Chemical Technology, University of Karachi, Karachi, 75270, Pakistan
[b] H.E.J. Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan

ARTICLE INFO

Keywords:
Silver nanoconjugates
Escherichia coli
Cefaclor
AFM
MTT assay
Cu(II)-doped *Escherichia coli*

ABSTRACT

E. coli strain is a gram-negative bacterium known to induce both extra-intestinal infections and intestinal infections. For survival of microbes, metal intake and accessibility should be according to their physiological requirements. Peculiarly, copper homeostasis is critical for *E. coli* survival and growth. Therefore in this study, an extensive work is conducted to investigate the impact of Cu(II)-doping on the susceptibility of *Escherichia coli* ATCC 10536 against Cu(II)-selective Cefaclor-silver nanoconjugates (i.e., Cf-AgNPs) and its organic precursor (i.e. Cefaclor). At first, the maximal non-cytotoxic dose of Cu(II) that was sub-lethal for *Escherichia coli* was determined by MTT assay and was found to be 100 μg/L. Afterwards, MICs of Cf-AgNPs and Cefaclor against controlled and Cu(II)-doped *E. coli* cells were determined by using Agar well diffusion method. The susceptibility of *E. coli* cells against Cf-AgNPs was increased upon Cu(II) doping, whereas the bactericidal activity of Cefaclor against Cu(II)-doped *E. coli* cells was retarded due to hydrolysis. In addition, morphological changes induced in controlled and Cu(II)-doped samples of *E. coli* after treatment with Cefaclor and Cf-AgNPs were also monitored by Atomic force microscopy (AFM). The obtained results from both Agar well diffusion method and AFM confirmed that Cf-AgNPs are more effective against Cu(II)-doped *Escherichia coli*. Moreover, thermal profile of Cu(II)-selective Cf-AgNPs was also demonstrated by TGA and DSC. This study can be an important part of the relevant state-of-the-art. Indeed, further clinical studies are necessary to determine the relevant role of Cf-AgNPs compared with that of the Cefaclor now available.

1. Introduction

It is critical for survival of microbes to make certain that metal intake and accessibility is in agreement with their physiological requirements, as disproportioning is injurious. Elevated intercellular concentration of transition metals is perilous, as they disturb the redox potential of cell (Porcheron et al., 2013). Indeed, host resistance mechanism against pathogenic bacteria either consist of metal malnourishment by producing proteins that sequester essential metals needed by microbes or by inducing virulence via excess liberation of metal cations. For instance, host discharges excess quantity of Cu(II), as proteins exhibit preferential selectivity towards copper even if identical amounts of all bivalent metal cations are available (Waldron and Robinson, 2009). Preferential selectivity of bacterial proteins towards excess Cu(II) is perilous as bacteria utilize Cu(II) as a catalyst for electron transfer reactions due to its unique redox potential. Therefore, owing to its toxicity, intracellular Cu(II) level should be delicately controlled. To overcome the resistance offered by the host, bacteria utilize import and export systems, and delicately normalize metal homeostasis by various transcriptional regulators, authorizing them to acclimatize to altering ecological circumstances.

E. coli strain is a gram-negative bacterium and can induce both extra-intestinal infections (induced via extra-intestinal pathogenic *E. coli* [ExPEC]) and intestinal infections (induced conjointly via disparate intestinal pathogenic *E. coli* [IPEC]). Cu(II) resistance observed in Gram-negative bacteria implied three distinctive mechanisms: (1) Transporting Cu(II) from cytoplasm towards periplasm by means of P1B-type ATPases, (2) Removal of extra Cu(II) via CueO multi copper oxidase and (3) Eradicating unreacted periplasmic Cu(II) either by its disposition or confiscation (Dupont et al., 2011; Hodgkinson and Petris, 2012).

This study is designed to investigate the effect of Cu(II)-doping on the vulnerability of *Escherichia coli* against Cefaclor and self-assembled Cu(II)-selective Cefaclor-silver nanoconjugates (Cf-AgNPs). In addition,

Abbreviation: Cf-AgNPs, Cu(II)-selective Cefaclor-silver nanoconjugates

* Corresponding author at: Department of Applied Chemistry and Chemical Technology, University of Karachi, Karachi, 75270, Pakistan.
E-mail address: wasiarasheed7@gmail.com (W. Rasheed).

https://doi.org/10.1016/j.micron.2018.05.002
Received 21 March 2018; Received in revised form 2 May 2018; Accepted 3 May 2018
Available online 08 May 2018
0968-4328/

the maximal non-cytotoxic dose of Cu(II) was determined by MTT assay. Afterwards, cell integrity of *E.coli* after doping with maximal non-cytotoxic dosage of Cu(II) was monitored by AFM. Moreover, the susceptibility differences between controlled and Cu(II)-doped cells were determined by Agar well diffusion method. Furthermore, the morphological changes induced in the resultant strains (i.e. controlled and Cu(II)-doped *E. coli*) after treatment with Cefaclor and Cf-AgNPs were investigated by AFM.

2. Materials and methods

2.1. Materials

Silver nitrate ($AgNO_3$) was purchased from Merck. Sterilized water was used as a solvent for the preparation of Cu(II)-selective Cefaclor-silver nanoconjugates and further analysis.

2.2. Synthesis of Cu(II)-selective Cefaclor-silver nanoconjugates (Cf-AgNPs)

Cf-AgNPs was formulated by reducing Ag^+ via Cefaclor, already reported (Rasheed et al., 2016).

2.3. Imaging substrate and bacterium specimen

Choice of imaging substrate rely on its surface charge, hydrophobicity, and ultra-flat film forming capability, muscovite therefore was selected, as it possesses all above stated attributes (e.g. point-of-zero-charge (pzc) equivalent to 2 to 3, contact angles (θ) equivalent to near 0° and perfect basal cleavage) (Adamson and Gast, 1997; Stumm and Morgan, 1996). In vitro bactericidal activities of test compounds were estimated against *Escherichia coli* ATCC 10536.

2.4. Determination of threshold value of Cu (II) by MTT assay

Seeded lawn of freshly cultivated *E. coli* (having a concentration equivalent to 10^6 cells in one mL) was dispensed in every well of 96 wells plate. By utilizing Brain-heart infusion medium (BHIM) as a diluent, distinct solutions of Cu(II) (500 to 5 μg/mL) were formulated via serial dilution method. Subsequently, 200 μL of these distinct solutions of Cu(II) was dispensed in discrete wells and incubation was conducted at 35.5 °C for 18–24 h. Afterwards, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide possessing a concentration of 5 mg/mL was dispensed in each well and continued the incubation for further 4 h by raising the temperature to 37 °C. After this incubative period, 200 μL of dimethyl sulfoxide was dispensed in each well and absorbance of each specimen was monitored at a primary wavelength of 570 nm and a reference wavelength of 650 nm (Wang et al., 2010).

2.5. Attachment factor for morphological studies

Conspicuous negative charges that dominate on the surfaces of both *E. coli* and muscovite form the oppositively charged α-poly(L-lysine) (PLL) an ideal attachment factor. Consequently *E. coli* remain attached to the muscovite even after the application of lateral forces exerted by AFM's tip. Moreover, it is gentle towards bacteria as PLL precursor amino acid occurs naturally and larger minimum inhibitory concentration of PLL was proclaimed (Conte et al., 2007). For adequate thickness of attachment factor, dry coating method was applied (Atabek et al., 2008; Bolshakova et al., 2001; Vadillo-Rodríguez et al., 2004).

2.6. Morphological studies by atomic force microscopy

For morphological studies, 5–10 μL droplets of specimens (having concentration equivalent to 10^6 cells in 1 mL) were dispensed on the PLL coated muscovite and left to dry. Afterwards, morphological studies were conducted by Agilant-5500 atomic force microscope, operated in tapping mode.

2.7. Minimum inhibitory concentration by Agar well diffusion method

Minimum inhibitory concentration of test compounds was determined by Agar well diffusion method (Nathan et al., 1978). Mueller Hinton Agar was utilized as a growth medium and distinct Agar plates of controlled and Cu(II)-doped *E. coli* cells were formulated at the concentration of 10^6 cells in one mL for testing against Cf-AgNPs and Cefaclor. The 200 μg/mL stock solutions of Cefaclor and Cf-AgNPs were serially diluted to avert nonspecific inhibition zones. In each well, various concentrations of test compounds (i.e., 150–5 μg mL^{-1}) were added. For uniform diffusion of test compounds, all plates were incubated at room temperature for two hours. Subsequently, these respective samples were incubated at 37 °C ± 1 for 48 h. After completion of incubation, inhibition zones of respective samples were assessed by utilizing a millimeter scale.

2.8. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

The melting point (Tm) of Cefaclor and amorphous to crystalline transition of Cf-AgNPs was determined in non-hermetically sealed aluminum pans, heated at a rate of 10 °C/min in DSC (Perkin Elmer DSC 6) using nitrogen as the purge gas and indium as the calorimetric standard. Cf-AgNPs was also subjected to thermogravimetric analyzer (Hi Res TGA 2950, TA). Weighed sample was heated in a platinum pan at a rate of 10 °C/min under nitrogen purge.

3. Results and discussion

Silver content of Cf-AgNPs analyzed by Thermogravimetric Analysis equals to 41.54% (Fig. 1). Owing to the evaporation of water adsorbed on the periphery of Cf-AgNPs, weight losses nearly 1 to 7% were observed in the range of 30–100 °C (Fig. 1). In the range of 150–225 °C, a little weight loss was also observed (Fig. 1) that is due to desorption of physically adsorbed Cefaclor (Rai et al., 2010). Major weight losses observed in the region of 225–625 °C were eventuated by the cleavage of coordinate covalent bonds between Cefaclor and silver (Fig. 1) (Rai et al., 2010).

Ascribable to the melting point, the obtained DSC curve of Cefaclor showed transition at 175.23 °C (Fig. 2(a))(Chow et al., 1998) and an exotherm in the DSC curve of Cf-AgNPs depicted that the transition from amorphous to crystalline state occurred at 345.10 °C (Fig. 2(b)) (Salkar et al., 1999).

For evaluation of Cu(II)-doping's impact on the susceptibility of *E. coli*, quantification of maximal non-cytotoxic dose of Cu (II) measured by MTT assay was imperative. Threshold value of Cu(II) for *E. coli* was determined to be 100 μg/mL because at and below this dosage *E. coli* assimilated Cu(II) completely as nature has endowed *E. coli* with effective Cu(II) handling systems. Recognized copper-reliant proteins in *E. coli*, besides copper homeostasis factors such as CueO, comprise the periplasmic zinc, copper SOD (SodC), cytochrome oxidase (CytBO (3)), NADH dehydrogenase-2 (NDH-2), 3-deoxy-Darabino-heptulosonate-7-phosphate synthase (AroF) and aromatic amine oxidase (MaoA) (Gort et al., 1999; Jordan et al., 2001; Osborne et al., 1999; Rapisarda et al., 2002). Thus, *E. coli* needs copper to establish appropriate redox potential within itself. Penetration of Cu(II) within *E. coli* was enabled via its outer membrane. Subsequently, it accessed the periplasm through porins. Afterwards, reduction of cupric to cuprous was facilitated by cupric reductase NDH-2, since copper is permitted to travel across the cytoplasmic membrane only in its reduced state (Outten et al., 2000; Rapisarda et al., 2002; Rapisarda et al., 1999). After reaching the cytoplasm, *E. coli* utilized copper as per its cellular needs and the effluxion of surplus copper was controlled via CopA, as it is a key constituent of