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Jacalin-copper sulfide nanoparticles complex enhance the antibacterial activity against drug resistant bacteria via cell surface glycan recognition

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ABSTRACT

In any therapeutic modality the usage of drug in high doses often leads to serious side-effects. Herein, we demonstrated a method to enhance the antibacterial efficacy of CuS NPs at lower concentration through interacting with jackfruit seed lectin, jacalin. Fluorescence quenching studies revealed that jacalin form complex with CuS NPs and the association constant was $1.91 \times 10^4\ M^{-1}$. Upon complex with jacalin, the bacterial minimum inhibitory concentration (MIC) of CuS NPs drastically decreases from 12.5 μM to 0.78 μM. The addition of jacalin specific sugar, galactose to jacalin-CuS NPs complex (JCuS NPs) reverses the MIC from 0.78 μM to 25 μM. Mechanistic study suggests that JCuS NPs kills bacteria in part by reactive oxygen species and membrane damage, but galactose prevents the action of JCuS NPs at 0.78 μM. JCuS NPs successfully reduce (14 fold) *A. hydrophila* colonization in an infected zerbra fish and rescue them completely from the infection, but galJCuS NPs and CuS NPs were ineffective at 0.78 μM. Collectively, our studies demonstrates that the enhance antibacterial activity of JCuS NPs is likely due to the interaction between the galactose binding site of jacalin and the bacterial strains, as a result NPs are targeted and delivered sufficiently.

1. Introduction

Infectious diseases caused by the microorganisms are the major threat to the global health care. Recent statistics suggest that 47% of hospital deaths are caused by infectious pathogens [1]. For example, approximately 1.4 million deaths have been reported due to tuberculosis in 2015 [2]. Bacterial infections are commonly treated with antibiotics, which are known to target the growth process of the bacteria and damages the cellular structure of microorganisms [3]. Besides the benefits, the repeated use of the antibiotics over the years generated drug resistance in the bacteria which becomes a major problem in antibiotic therapy [4]. These bacteria develop antibiotic resistance majorly through efflux pumps, biofilm formation, retarded outer membrane permeability and mutation [5,6]. The available medical records showed that the treatment of drug-resistant bacterial strains require a high-dose administration of antibiotics, usually multiple expensive drugs which in general are less effective and more toxic and often develops undesirable side effects [7]. Therefore, there is a growing demand for an alternative strategy to treat bacterial infections with no or minimal side effects along with the lack of resistance development on the part of the pathogen.

In recent years, metals and metal oxide nanoparticles such as Au, Ag, Cu, Pt, Pd, ZnO, TiO_2, Bi_2O_3, CuO, and Fe_2O_3 etc. are reported to have excellent antibacterial properties [8–11]. For example, AgNPs exhibit excellent antibacterial activity and have been used in diverse areas such as for the production of clothing, catheters, electric home appliances and biomedical implants [12]. However, AgNPs displays high toxicity in vivo [13]. CuNPs too exert strong antibacterial activity against both Gram positive and Gram negative pathogens; however it exhibits high toxicity in vivo [14]. Nevertheless, while converting CuNPs into copper sulfide nanoparticle (CuS NPs), the toxicity decreases drastically [15] and also displays good antibacterial activity [16]. CuS NPs have been emerging as a promising platform for photothermal cancer therapy, biomolecule sensing, and molecular imaging [17–19].

In our continuous effort in developing metal nanoparticles to combat bacterial infections, we report a potential method to enhance the antibacterial activity of CuS NPs at lower dosage through interacting with jacalin. Jacalin is 66 kDa protein isolated

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from the seeds to jackfruit [20]. It binds to various glycans, including galactose, N-acetylgalactosamine, mannose, N-acetylmuramic acid and N-acetylneruamic acid [21]. The bacterial cell wall is composed of a wide array of glycans and forms a major protective barrier for the bacterium, thus, becoming an important target for any new antibiotics [22]. Having known that jacalin binds to glycans and the bacteria cell wall is made of glycans, we surmised that jacalin-drug complex may have better antibacterial properties than the free drug. To test this hypothesis, first we studied the interaction between jacalin and CuS NPs by fluorescence spectroscopy. The results obtained from the study revealed that jacalin binds to CuS NPs with an association constant of 10^{-4} M^{-1}, which is comparable to lectin-carbohydrate interaction [23,24]. As prepared non-covalent jacalin-CuS NPs (JCuS NPs) complex displayed excellent antibacterial activity against Gram positive and Gram negative bacteria. While complex with jacalin, the minimum inhibitory concentration of CuS NPs drastically decreased from 12.5 μM to 0.78 μM against all the tested bacterial strains. Inspired from this result, the antibacterial efficacy of JCuS NPs was assessed against drug resistant clinical isolates of Methicillin resistant Staphylococcus aureus (MRSA). Interestingly, the JCuS NPs inhibited the growth of the MRSA strains also at a reduced MIC. The antibacterial mechanistic study revealed that jacalin-CuS NPs exert bactericidal activity through glycan recognition and kills the bacteria through the generation of reactive oxygen species and membrane damage.

2. Experimental

2.1. Materials

Copper chloride, hydrazine, ammonium hydroxide and sodium sulfide were obtained from Merck, India. Tyramine, laurylchloride, resazurin, dichlorofluorescein diacetate, acridine orange, ethidium bromide, 5,5′ dithio-bis(2-nitrobenzoic acid), acetylcholine iodide, naphthyl ethylenediamine hydrochloride were purchased from Sigma, India. All microbiological media were obtained from Himedia, India. *Bacillus subtilis* (MTCC441), *Staphylococcus aureus* (MTCC3160), *Escherichia coli* (MTCC723) and *Aeromonas hydrophila* (MTCC1739T) were obtained from Institute of microbial technology, India. The MRSA reference strain (ATCC 33591) and the three clinical strains of MRSA (GSA-45, GSA-395 and GSA-410) were generously gifted by Dr. S. Karutha Pandian, Alagappa University, India. The organisms were preserved at 4 °C and sub-cultured at regular intervals of 30 days. All other chemicals and reagents were of the highest analytical grade and commercial available.

2.2. Purification of jacalin

Jacalin was purified by affinity chromatography on cross-linked guar gum as described previously [24]. The eluting sugar galactose was removed from jacalin through dialysis against 10 mM sodium phosphate buffer, pH 7.4, containing 150 mM sodium chloride (PBS). The protein purity was judged by polyacrylamide gel electrophoresis in the absence as well as in the presence of sodium dodecylsulfate. The concentration of jacalin was determined by Lowry assay using bovine serum albumin as the standard [41].

2.3. Interaction of copper sulfide nanoparticles with jacalin

N-lauryltyramine (NLTA) capped copper sulfide nanoparticles were prepared according to a previously reported method [16]. Briefly, 1 mM NLTA capped copper nanoparticles were directly allowed to react with 1 mM sodium sulfide for 3 h. The formation of CuS NPs was evident from the color change from wine red to green. The interaction between jacalin and CuS NPs were monitored in a Jasco-FP8200 spectrofluorimeter. The intrinsic fluorescence spectra of jacalin were recorded in 300–400 nm at an excitation wavelength of 280 nm. The spectral slit width was set to 5 nm for both excitation and emission monochromators. A fixed volume of jacalin solution (3.0 mL, 2.5 μM) was titrated by adding small aliquots of the CuS NPs from a concentrated stock solution (0.5 mM) and the fluorescence intensity was recorded after an equilibration period of 2 min. To determine the binding of CuS NPs interferes with the natural saccharide binding characteristic of jacalin, we performed NPs interaction studies by pre-incubating jacalin with a high concentration (50 mM) of galactose. All binding experiments were performed in PBS (phosphate buffered saline) buffer. All titrations were repeated at least three times to arrive at average values. Fluorescence intensities were corrected for volume changes before further analysis.

2.4. Lectin activity assay

Jacalin activity was checked by hemagglutination and hemagglutination inhibition assays as described in Ref. [30]. To determine whether CuS NPs binding, altered the sugar-binding activity of the lectin, the hemagglutination experiments were conducted by pre-incubating jacalin with 50 mM galactose.

2.5. In vitro antibacterial activity of jacalin-CuS NPs complex

The antibacterial activity of JCuS NPs were evaluated against Gram-positive (S. aureus, B. subtilis) and Gram-negative (E. coli, A. hydrophila) strains. The bacterial strains were cultured aerobically for 12 h at 37 °C in Luria-Bertani (LB) medium. The cultures were maintained by streaking on LB agar plates and were incubated at 37 °C for 24 h. All the MRSA strains were grown and maintained on Soybean casein digest agar/broth (SCD agar/broth (HiMedia, India). All the strains were grown to an optical density at 660 nm (OD_{660}) of 0.5-0.6 for experimental purposes.

The minimum inhibitory concentration (MIC) of JCuS NPs was determined by serial dilution method [31]. Typically, 100 μL of JCuS NPs (100 μM) was added to the 96-well plate and serial diluted and the final volume was made up to 100 μL using LB medium. Then, 100 μL of 1×10^5 cfu/mL bacterial cells were seeded into each well and cultured under shaking for 24 h at 37 °C. The OD_{660} at each well was monitored in microtiter plate reader (Thermo Scientific Multiscan EX). Further, the cell viability was determined by adding 30 μL of resazurin solution (0.01% wt/vol) to each well and cultured for 2 h at 37 °C. The wells with viable cells showed color change from blue to pink and exhibit strong fluorescence [32]. The fluorescence intensity in each well was monitored in a fluorescencemicroplate reader (Biotech, synergy H1, Japan), setting the excitation and emission at 530 nm and 580 nm, respectively. The MIC of CuS NPs and JCuS NPs containing 100 mM galactose was estimated using the above described procedure. The efficacy of JCuS NPs was further judged by the zone of inhibition (ZOI) assay. Log phase bacteria cells were swabbed uniformly on LB agar plates using sterile cotton swabs. Wells of 10 mm diameter were made on the plates using gel puncture. Using micropipette, 30 μL of 0.78 μM of NPs were added to the respective well and incubated at 37 °C for 12 h. The formation of the zone around the well confirms the antibacterial activity of NPs.

2.6. Scanning electron microscopy imaging

The changes in the bacteria morphology of the control (buffer treated) and JCuS NPs treated cells was analyzed by scanning electron microscopy (SEM) (Tescan Vega 3). For SEM sample preparation, glass slides were placed into the LB media either contains bacteria (1×10^5 cfu/mL) or bacteria with 0.78 μM JCuS NPs. After 12 h, the glass slides was carefully removed from the media and