Charge-directed targeting of antimicrobial protein-nanoparticle conjugates

Rohan Satishkumar and Alexey Vertegel.
Clemson University, Department of Bioengineering

Statement of Purpose: Use of enzymes as antimicrobial agents is nature-inspired and has recently attracted much attention as an antibiotic-free approach to treat bacterial infections [1]. Use of antibacterial enzymes covalently attached to nanoparticles is of special interest because of enhanced stability of protein-nanoparticle conjugates and the possibility of targeted delivery. Intrinsic properties of nanoparticles can also be used to add functionality to their conjugates with biomolecules. Here, we show in a model system that nanoparticle charge can be used to enhance delivery and increase bactericidal activity of an antimicrobial enzyme, lysozyme.

Methods: Preparation of protein-nano conjugates: Hen egg white lysozyme (HEWL) from Sigma was covalently attached to two types of latex nanoparticles: positively charged containing aliphatic amine surface groups and negatively charged containing sulfate and chloromethyl surface groups. Polystyrene latex nanoparticles (20 nm, Molecular Probes) were used for the conjugation. In the case of protein attachment to aminated particles, glutaraldehyde was used as the coupling agent followed by reduction by sodium cyanoborohydride. Attachment to chloromethyl groups was carried out by direct interaction with the amine groups of HEWL in MES buffer at pH=6.0. Tween 20 (0.1 wt. %) was added to remove physically adsorbed enzyme and, the samples were centrifuged to separate any unbound protein.

Binding efficiency studies were performed to measure the amount of protein bound to the nanoparticles using BCA™ and Micro BCA™ assay kits (Pierce Biotechnology, Rockford, IL). Protein-nanoparticle conjugates were characterized by dynamic light scattering and zeta potential measurements.

A bacterial turbidometric activity assay was performed by monitoring the decrease in optical density of an aqueous suspension of *Micrococcus lysodeikticus* in potassium phosphate buffer [2]. An alternative assay with a neutral chromogenic substrate, *p*-nitrophenyl-penta-*N*-acetyl-*β*-chitopentaoside, PNP-$(GlcNAc)_5$, was performed by monitoring the change in absorbance at 405 nm [3].

Results/Discussion: In the case of the bacterial degradation assay, activity of lysozyme attached to positively charged nanoparticles was higher than that of free lysozyme, whereas lysozyme attached to negatively charged nanoparticles showed no detectable activity (Fig 1). When colorimetrically assayed using a neutral oligosaccharide substrate PNP-$(GlcNAc)_5$, lysozyme attached to both positively and negatively charged nanoparticles showed similar activity to that of the free enzyme (Fig 2).Bacterial cell walls are negatively charged due to the presence of teichoic acids covalently linked to the peptidoglycan layer. Lysozyme attached to negatively charged nanoparticles cannot be effectively targeted to its substrate because of the electrostatic Coulombic repulsion with the cell wall, while lysozyme conjugated to

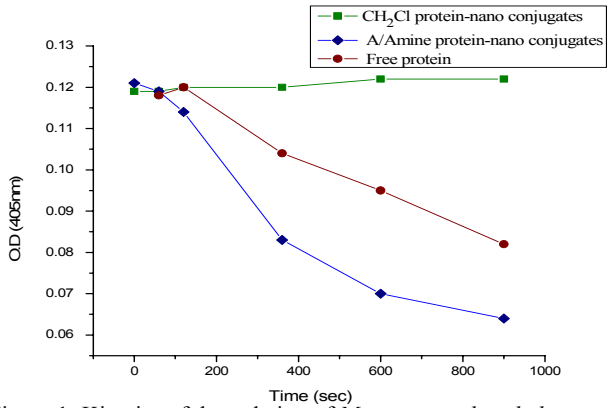

Figure 1: Kinetics of degradation of *Micrococcus lysodeikticus* by free lysozyme (circles) as compared to that of the lysozyme attached to positively (diamonds) and negatively (sqaures) charged nanoparticles.

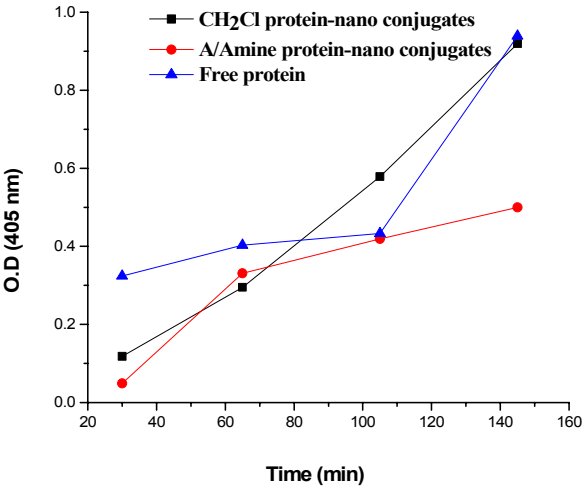

Figure 2: Activity of lysozyme conjugated to both negatively and positively charged nanoparticles in the reaction with an uncharged substrate is similar to activity of free lysozyme

positively charged nanoparticles is targeted more effectively than free lysozyme. On the contrary, when the neutral PNP-$(GlcNAc)_5$, substrate is used, attachment to nanoparticles and nanoparticle charge do not show a considerable effect on the enzymatic activity. Thus, nanoparticle charge is an important factor that can be used to control activity of protein-nanoparticle conjugates.

Conclusions: Charge-directed targeting of antimicrobial protein-nanoparticle conjugates was achieved using positively charged nanoparticles. Targeting of these protein-nanoparticle conjugates to the bacterial cells was more efficient than that of free enzyme.

References:

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