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Bioanalytical Assay of Antimicrobial Polymers Binding to Bacterial Cells

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Abstract

Branched polyethylenimine (BPEI) has an antimicrobial effect on bacteria. The killing mechanism of BPEI centers on its cationic properties. The mechanism of action against Gram-positive bacteria is less understood but recent reports erroneously suggest that membrane depolarization occurs. To the contrary, data from our laboratory suggests that BPEI binds to the anionic sites provided by the biopolymer wall teichoic acid (WTA). To test the validity of this hypothesis, we measure the amount BPEI binding to whole, intact, bacterial cells of *Bacillus subtilis*. Comparative measurements are made with *Bacillus subtilis* bacteria that contain WTA and *Bacillus subtilis* genetic mutants that lack WTA.

Using equilibrium dialysis, *Bacillus subtilis* bacteria were exposed to different solution concentrations of BPEI. Removal of small aliquots from solution and subsequent assay with the ninhydrin test were used to measure the amount of BPEI remaining in solution and the amount of BPEI bound to the bacterial cell walls. These data were used to obtain the amount of bound vs. unbound BPEI and determine the equilibrium constant. These data influence the understanding of BPEI antimicrobial properties and impacts the development of antibiotics to treat human disease.

KEYWORDS: Branched polyethylenimine (BPEI); Bacillus subtilis; wall teichoic acid (WTA); equilibrium dialysis

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Branched polyethylenimine (BPEI) has an antimicrobial effect on bacteria. The killing mechanism of BPEI centers on its cationic properties. The mechanism of action against Gram-positive bacteria is less understood but recent reports erroneously suggest that membrane depolarization occurs. To the contrary, data from our laboratory suggests that BPEI binds to the anionic sites provided by the biopolymer wall teichoic acid (WTA). To test the validity of this hypothesis, we measure the amount BPEI binding to whole, intact, bacterial cells of *Bacillus subtilis*. Comparative measurements are made with *Bacillus subtilis* bacteria that contain WTA and *Bacillus subtilis* genetic mutants that lack WTA.

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