New and Notable

A Chaotic Pore Model of Polypeptide Antibiotic Action

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In this issue of Biophysical Journal, Gregory et al. present a new model for the action of cecropin A on synthetic lipid vesicles (1). Cecropin A was one of the first polypeptide antibiotics identified by Boman and co-workers (2) following his observation that antimicrobial activity could be induced in insects by bacterial infection (3). Since then, many other ribosomally synthesized antibiotics have been identified in both plants and animals, where it has become clear that they are important in diverse ways to host defense. When expressed in transgenic animals, for example, they can enhance host defenses against bacterial infection (4). Human deficiency states, on the other hand, lead to an increased incidence of infection (5,6) and inflammatory bowel disease (7).

Despite thousands of articles about hundreds of these polypeptide antibiotics over several decades, basic questions about their mechanism of action remain unanswered. Most investigators have concluded that they act solely by permeabilizing the bacterial cell membrane. Although strong evidence for another mechanism of action has not yet emerged, this conclusion may not represent the whole story because polypeptide antibiotics clearly have other significant effects on bacteria (8–11). Moreover, few naturally occurring antibiotics have only one mechanism of action, presumably because antibiotics with multiple mechanisms of action have been selected to overcome the development of resistance to any one mechanism. Resistance to polypeptide antibiotics is common, and different polypeptides tend to have different patterns of resistance or susceptibility among bacteria. Resistance to any one polypeptide often varies widely among closely related bacterial species, and can even vary among different strains of the same species.

It is difficult to explain these diverse patterns of resistance with a single mechanism of action targeting lipids in the bacterial cell membrane. Of course, resistance may occur when access to the site of action is blocked. This explanation may account for the resistance observed in one case where changes in growth medium induced changes in the outer membrane (i.e., not the cell membrane) of a Gram-negative bacterium (12). However, if polypeptide antibiotics must overcome selective barriers to reach their site of action, any description of their mechanism of action must be expanded to include this capability.

In any case, bacteria do not develop secondary resistance when cultivated in subinhibitory concentrations of polypeptide antibiotics, as is usually the case with antibiotics in other classes. This ''resistance to acquired resistance'' has drawn considerable attention among investigators seeking new antibiotics for use against the growing menace of pathogens with multidrug resistance. Moreover, most animals produce an assortment of polypeptide antibiotics. These factors may have allowed polypeptide antibiotics to flourish in nature despite having only a single mechanism of action.

Given the complex and fundamental questions that persist about their mechanism of action in bacteria, one must be circumspect when drawing conclusions about polypeptide antibiotics from studies of their effects on synthetic lipid vesicles. Vesicles do not have the complex composition or structure of bacterial membranes, and it isn't clear whether they more closely resemble the membranes of bacteria that are susceptible or resistant to a particular polypeptide antibiotic. Yet because of their simplicity, synthetic vesicles facilitate quantitatively rigorous investigations into the interactions of polypeptides with membranes.

The model of Gregory et al. is based solely on the interactions of cecropin A with synthetic lipid vesicles, but it is nonetheless impressive in several respects. First, and most notably, the model is quantitatively elegant. It accounts for the kinetics of vesicle contents release with a single adjustable parameter, β. Second, this parameter has a clear physical interpretation, being the ratio of the rates of formation and relaxation of a ''pore state.'' Third, the model provides key insights into mechanism of action while discriminating against alternative models.

The mechanism of action suggested by the model of Gregory et al. is that polypeptides antibiotics induce the transient existence of a chaotic pore state by creating structural distortions and tensions when they situate in a lipid bilayer. Carefully measured on- and off-rates appear to exclude earlier versions of this mechanism involving polypeptide translocation across lipid bilayers (13, 14). They also weigh against the toroidal pore model, and there is no need for an organized structure of this type in Gregory's model. The relatively sparse surface coverage needed to create the pore state, and the restoration of polypeptide binding kinetics after relaxation of the pore state, both weigh against the popular carpet model.

The nascent model of Gregory et al. is now obliged to run a gauntlet of challenges. First among these challenges is a demonstration that it applies to polypeptides other than cecropin A. Information about β for a series of antibiotics may provide insight into the features of polypeptide sequences that account for their antibiotic activity.

Submitted November 13, 2007, and accepted for publication December 4, 2007.

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Editor: Edward H. Egelman.

0006-3495/08/03/1549/02 $2.00

doi: 10.1529/biophysj.107.124792

Additional important challenges include a demonstration that the effects of polypeptide antibiotics on vesicles faithfully represent what happens on bacterial membranes, and an explanation for their differential activity against prokaryotic versus eukaryotic membranes.

It is commonly assumed that cationic polypeptide antibiotics like cecropin A act against bacteria and not eukaryotic cells because bacteria have anionic lipids on the outer surface of their cell membranes. However, there is little experimental support for this assumption. Gram-negative bacteria have more protein than lipid in their cell membranes, anionic lipids are a minority component among the lipids that are present (15), and we do not know how they are distributed between the inner and outer surfaces of the cell membrane—there is simply no data. A much higher fraction of lipid in Gram-positive bacteria is anionic, but these bacteria are not more susceptible to polypeptide antibiotics. Gregory et al. observed that anionic lipids have relatively little effect on β, suggesting that they are not directly involved in membrane permeabilization (16). On the other hand, anionic lipids did influence the amount of polypeptide bound to the vesicles. If this effect is due to nonspecific electrostatic interactions, then other more abundant anions (e.g., polysaccharides or membrane proteins) may have a greater role than anionic lipids in binding and concentrating polypeptide antibiotics on the bacterial surface.

Whether or not Gregory's model survives the challenges it now faces, one may hope that its initial success stimulates further quantitative investigation into a host defense mechanism that is so broadly applied in nature.

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