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Nafsika H. Georgopapadakou

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Bacterial Transport as an Import Mechanism and Target for Antimicrobials

Robert E. W. Hancock
University of British Columbia, Vancouver, British Columbia, Canada

I. INTRODUCTION

In the past 50 years, literally tens of thousands of antibacterial compounds have been chemically synthesized or isolated from soil microorganisms, plant, aquatic, or other natural sources, and systematically modified. However, it is becoming clear that, as rapidly as compounds are developed, subsets of bacteria are developing resistance.

To my knowledge, no fundamentally new, useful antibiotic structures have been developed in the past 25 years, with the possible exception of the cationic peptides (1). In the search for clinically useful compounds, two strategies hold significant promise. One is to devise methodologies that can be employed to increase uptake by overcoming the intrinsic impermeability of bacterial cells toward potential antimicrobial chemicals. Such a strategy, involving piggybacking on natural bacterial transport systems, is considered in Section II. A second strategy involves the identification of novel targets for antibiotics (e.g., transport; see Sec. III) that can be used to devise targeted screens for the identification of novel compounds.

A. Barrier Function in Bacteria

Generally speaking, antimicrobials can be divided into those that are selective for gram-positive bacteria, those that have superior activity against

gram-negative bacteria, and the broad-spectrum antimicrobials. Although certain bacteria represent special cases, for example, mycobacteria (see Chap. 9), mycoplasma, and ureaplasma, and selective antibiotic-resistant organisms (e.g., *Xanthomonas maltophilia*), these antibacterial specificities can usually be explained as follows. Antibiotics that are relatively selective for gram-positive bacteria tend to be excluded by the unique outer membrane of the gram-negative bacteria. For example, the poor susceptibility of gram-negative bacteria to vancomycin, bacitracin, erythromycin, the ionophores, rifampin, clindamycin, fusidic acid, penicillin G, methicillin, and novobiocin, can be simply explained by the barrier effect of the outer membrane (2). In contrast, the few antimicrobials with better gram-negative activity are generally those, such as polymyxin, the octapeptins, and certain of the cationic peptides, that interact with the outer membrane as the first step in their action on cells (3). The physiological basis for these two observations is described later (see Sec. II.A.3). Those compounds with equivalent activities against both gram-negative and gram-positive bacteria generally pass through the outer membrane efficiently [although not freely, since a 4- to 16-fold disparity in minimum inhibitory concentrations (MICs) is common]. Even these antibacterial compounds, however, can be rendered clinically less effective against gram-negative bacteria, such as *Pseudomonas aeruginosa*, with intrinsically poorly permeable outer membranes (3).

B. Substrate Transport Systems

Although not all bacteria grow at equivalent rates, many important pathogens can double their masses (and numbers) every 30 min–1 h. Similar rapid rates of mass doubling can occur in either optimized culture media or in vivo (4,5). To support such rates of mass increase, bacteria require efficient uptake mechanisms for a variety of nutrients, including a source of carbon, reducing equivalents, nitrogen, oxygen, phosphorus, sulfur, potassium, sodium, magnesium, iron, chlorine, and trace elements, including Co, Zn, Mo, Cu, and Mn, as micronutrients. All of these represent essential building blocks and must be imported by specific transport systems. Many of these transport systems have been well studied in *Escherichia coli* and other bacteria (6) and are described in overview here. With certain prominent exceptions (e.g., iron uptake, see later discussion), we know little about the actual chemical form of these elements that are utilized in vivo. Nor do we understand which transport mechanisms are actually employed by bacteria growing inside eukaryotic hosts. A further complication is posed by regulatory mechanisms in bacteria that optimize growth efficiently. For example, the presence in a bacterium's environment of a building block for macromolecular synthesis, often leads to down-regulation of metabolic synthesis (by allosteric end-product inhibition or transcriptional down-regulation of synthesis of key enzymes), followed by uptake of the building block from the environment. Such a transport system would not make a good target for inhibition, since loss of uptake would generally lead to a counterbalancing adjustment of metabolic activity. Similarly, when multiple alternative uptake systems exist (e.g., for specific carbon sources, since bacteria can generally use a range of carbon sources), there is little prospect for isolation of a transport inhibitor. With these prefacing remarks, it is worth briefly describing the nature of bacterial uptake systems.

There are three major classes of uptake systems: passive diffusion, facilitated diffusion, and active (energized) transport (7,8). A fourth class would be porin-mediated passive diffusion, whereby small hydrophilic substances cross the outer membranes of gram-negative bacteria through nonspecific, water-filled channels of proteins, termed porins. This is passive diffusion in the sense that it obeys Fick's law, although there are some restrictions to free diffusion owing to frictional, steric, and charge interactions (see Chap. 6). With this addendum to traditional classes of transport systems, a summary of bacterial transport systems is presented in Table 1. For a more detailed description, the reader is referred to specific reviews (7,8). In general, similar types of transport systems exist in eukaryotic host cells. However, the active transport of substrates is more common in bacterial cells, which require such concentrative mechanisms to permit growth at the usual rapid rate of bacterial doubling, whereas the nutrient supply of the cells of the complex eukaryotes is often accomplished through either facilitated diffusion or pinocytosis (7,8).

C. Known Targets of Antimicrobial Drugs

The targets of antimicrobial compounds must be such that their inhibition either causes growth cessation (*bacteriostatic compounds*) or loss of cellular integrity or ability to form colonies (*bactericidal compounds*). There are relatively few classes of targets for bactericidal antimicrobials (9). These include destruction of peptidoglycan, leading to osmotic lysis; loss of cytoplasmic membrane integrity; or irreversible damage to DNA, (including double-stranded breaks, certain base modifications, cross-linking, or presumptive loss of DNA membrane attachment. However, many bactericidal antibiotics have modes of action that are difficult to assign to a single, specific inhibitory step and may be quite complex (10). In contrast, the action of bacteriostatic drugs is often more easily defined and can involve any essential metabolic event in bacterial cells.

Table 1 Bacterial Transport Systems

Uptake mechanism	Description	Natural substrates	Antibiotic substrate (ref.)
Passive diffusion	Free movement across lipid bilayers	H_2O, N_2, NH_3	Hydrophobic antibiotics (2); fluoroquinolones; tetracyclines (45)
Porin-mediated passive diffusion	Diffusion through the aqueous channels of outer membrane porin proteins	Small hydrophilic or charged substrates (amino acids, ions, sugars)	β-Lactams (3,24)
Facilitated diffusion	Passage along a concentration gradient across a lipid bilayer membrane using a specific substrate-binding protein (e.g., specific porins in the outer membrane)	Varies among bacteria (e.g., maltose, nucleotides, phosphate, glucose, fatty acids, specific iron–siderophore complexes)	Imipenem (48); cathechol β-lactams (15,19); albomycin (9); albicidin (52)
Active transport	Concentrative uptake dependent on energy expenditure, and a substrate-binding protein (e.g., cytoplasmic membrane "carriers")	Amino acids, metal cations, sugars, nucleotides	Aminoglycosides (3); D-cycloserine (9); phosphomycin (9); alaphosphin (9)

II. KNOWN SUBSTRATE TRANSPORT PATHWAYS AS ROUTES OF ANTIBIOTIC UPTAKE

There are two categories of substrate transport systems that can be employed to increase uptake of antimicrobial compounds (Table 2). One is the promiscuous uptake systems. These involve transport systems that will take up a wide variety of compounds (within parameters that are presumably set by the physical constraints imposed by channel architecture) provided they contain a specific prosthetic group. The second, and perhaps less useful, category of transport systems is the narrow-specificity systems. These generally accept only closely related analogues.

Table 2 Known Substrate Transport Pathways as Routes of Antibiotic Uptake

Pathway	Location	Antibacterial transported
Ferrichrome	Outer membrane (FhuA)	Albomycin, rifamycin, CGP4832
Other iron uptake	Outer membrane	Ferrioxamine B, ferrimycin, ferrocin
Basic amino acids	Outer membrane	Imipenem
Nucleotide	Outer membrane (Tsx)	Albicidin
Self-promoted uptake	Outer membrane	Nourseothricin, melittin, cecropins, defensins, polymyxin, colistin, aminoglycosides, azithromycin, teicoplanin aglycones
Phosphate	Cytoplasmic membrane	Arsenate
Iron scavenging	Outer membrane (Cir, Fiu)	Catechol β-lactams (BRL 41897A, GR69153, E-0702)
Oligopeptide	Cytoplasmic membrane (Opp)	Compounds IV, V, VI; alaphosphin
Alanine	Cytoplasmic membrane	D-Cycloserine
α-Glycerophosphate	Cytoplasmic membrane	Phosphomycin
Peptide permease	Cytoplasmic membrane	Alaphosphin

A. Promiscuous Uptake System

1. *Catechol–Iron Complex Uptake*

Bacteria are obligately dependent on iron for growth. However, iron exists in nature largely in insoluble complexes and in host fluids and tissues complexed to transport proteins, such as transferrin and lactoferrin. Thus, the amount of freely available iron is minimal, and bacteria have evolved efficient means of capturing and importing ferric iron for use in redox enzymes and cytochromes. Generally speaking, three types of iron transport systems exist: siderophore–iron uptake, transferrin– or lactoferrin–iron uptake, and the scavenger systems.

Siderophore–iron uptake (11) involves the synthesis and secretion of compounds (siderophores) with high affinities ($K_a = 10^{21}$ M) for Fe^{3+}. Siderophores usually fit into one of two general classes of iron-binding core structures, hydroxamates or catechols. However, the prosthetic groups attached to the core structures can vary in such a way that they confer host specificity during uptake of the iron–siderophore complex.

This is accomplished in gram-negative bacteria by the synthesis of a specific, coregulated, outer membrane receptor protein (e.g., FepA for ferri-enterochelin uptake in *E. coli*) (11). Translocation across the outer membrane has been proposed to require an energized event involving the cytoplasmic membrane proton motive force and a cytoplasmic membrane protein TonB that spans the periplasm and contacts a region (the TonB box) of the outer membrane receptor. The bacterial components involved in siderophore synthesis and ferri–siderophore complex uptake and processing are up-regulated by iron deficiency, which is the normal growth condition of pathogenic bacteria in their host.

Siderophore–iron-uptake systems are usually quite specific. For example, the peptide catechol siderophores of fluorescent *Pseudomonas* species tend to be quite strain-specific (12). Nevertheless, at least one system, the iron–hydroxamate (ferrichrome)-uptake system, is flexible enough to permit uptake of analogues that have antibiotics attached. Thus, the semisynthetic rifamycin derivative CGP4832 913 and the ferrichrome analogue albomycin have MICs in iron-depleted medium of 0.02–0.005 μg/ml. However, mutations that prevent ferrichrome uptake, including loss of the outer membrane receptor FhuA, or of the energy transducing protein TonB, lead to MICs for both compounds of 8–16 μg/ml.

A second class of uptake systems involves the direct binding of transferrin– or lactoferrin–iron complexes to outer membrane receptor proteins on the surface of such bacteria as *Neisseria*, *Haemophilus*, *Pasteurella*, and others (14). The subsequent mechanisms involved in ferric iron uptake are poorly understood, and there are no known antimicrobial compounds that utilize this system.

The third class of iron-uptake systems involve the scavenger systems. In *E. coli*, in which these systems have been best studied, the relevant outer membrane proteins involved are Cir (the colicin I receptor) and Fiu (15,16). For example, these proteins can mediate uptake of iron complexed to dihydroxybenzoyl-serine, a degradation product of enterochelin (11). These proteins seem to be able to function in uptake of a broad range of β-lactam compounds with appended catechol substituents (15–18). As with the siderophore–iron-uptake systems, the scavenger iron-uptake systems are dependent on TonB and are up-regulated in low iron medium. Similarly, the catechol-β-lactams that utilize the scavenger pathway work preferably in low iron growth environments (i.e., host conditions) and are TonB-dependent.

2. *Oligopeptide Uptake*

Salmonella typhimurium and *E. coli* contain a promiscuous transport system for uptake of oligopeptides (*opp*; 19,20). The oligopeptide permease system transports di- to pentapeptides, although this may partly reflect the exclusion limit of the outer membrane. The system involves four proteins, a periplasmic-binding protein OppA of molecular weight 52,000, and three membrane-associated proteins OppB, OppC, and OppD (the latter being an ATP-binding protein). The genes for these proteins constitute an operon in both *E. coli* and *S. typhimurium* and are expressed constitutively. The oligopeptide permease system is quite promiscuous and has been used to promote uptake of certain phosphorylated intermediates (21,22) and cytidine monophosphate (CMP)-α-keto-3-deoxyoctanate (KDO) synthase inhibitors (compounds IV, V, and VI) (23). In addition, dipeptide-linked CMP-KDO synthase inhibitors were active against many *Enterobacteriaceae* and *Pseudomonas* species, suggesting that the *opp* uptake system is broadly distributed, although MICs of only 5–100 μg/ml were recorded, suggesting a certain minimal efficiency. Unfortunately, it appears that mutants lacking the system can be selected with high frequency. Thus, the oligopeptide permease route does not seem to be broadly useful for antimicrobial drug delivery.

3. *Self-Promoted Uptake*

The outer membranes of gram-negative bacteria are stabilized, in part, by divalent cation cross-bridging between adjacent surface-localized, negatively charged lipopolysaccharide (LPS) molecules (3,24,25). This explains the ability of the outer membrane to resist detergents and bile salts and to exclude hydrophobic compounds (3,24); chemicals that disrupt the cross-bridging, such as the divalent cation chelator EDTA, result in loss of barrier function for these compounds. Hancock et al. (26) proposed that the interaction of (poly)cationic antibiotics, such as aminoglycosides, at cross-bridging sites on LPS is the first step in "self-promoted uptake." This uptake route involves the initial interaction of polycations with the divalent cation-binding sites on LPS at the bacterial surface (3,37). Polycationic antibiotics, such as polymyxin B and the aminoglycosides, have affinities for these LPS sites that are two to three orders of magnitude higher than the native divalent cations (usually Ca^{2+} or Mg^{2+}) and, thus, can competitively displace them. Since the competing polycations are bulkier than the native divalent cations, they alter the outer membrane packing, resulting in blebs or transient cracks (3). This permits enhanced uptake of certain probe molecules, including the chromogenic β-lactam nitrocefin, the hydrophobic fluorophore 1-*N*-phenyl-1-naphthylamine (NPN), and the peptidoglycan-degrading enzyme lysozyme, across the permeabilized outer membrane (3). Therefore, it was hypothesized that polycationic antimicrobials promote their own uptake across the outer membrane, and the process was termed "self-promoted uptake."

Three major lines of evidence suggest that self-promoted uptake is relevant to eventual cell killing by polycationic antibiotics. First, outer membrane mutants that have reduced interaction with such polycationic antibiotics are resistant to killing by these antibiotics (27), whereas those with enhanced interactions are hypersusceptible (28). Second, for the aminoglycosides, there is a linear relation between the affinity of different aminoglycoside antibiotics for the cell surface and the MIC (29). Third, excess divalent cations that inhibit the interactions of polycationic antibiotics with the cell surface increase the MIC (3,29). With this in mind, self-promoted uptake has been demonstrated for a wide variety of polycations, including polymyxins, aminoglycosides, the macrolide azithromycin, teichoplanin aglycones, nourseothricin (streptothricin), and several cationic peptides, in a range of gram-negative bacteria (1,3,30–32).

The ability of polycations to promote their own uptake across the outer membrane as well as to promote the uptake of other probe molecules suggests two potential methods of improving antibiotic uptake into gram-negative bacteria. The first method for utilizing self-promoted uptake is to enhance the cationic character of the antibiotic in question. Two clear examples exist in the literature. The dibasic macrolide azithromycin was created chemically from the monobasic macrolide erythromycin by expansion of the 14-membered ring to include one extra methylamine with a positive charge. Azithromycin had substantially improved activity against *E. coli* (33) and appeared to be taken up by self-promoted uptake (31). In preliminary studies, a tribasic analogue had even better activity against gram-negative bacteria. The second example involved the glycopeptide antibiotic teicoplanin that has three negative charges and one positive charge and no useful activity against *E. coli* or *P. aeruginosa*. Removal of the sugar moieties deleted the negative charges and led to some anti-*E. coli* activity, whereas modifications at carbon 56 to add polyamines resulted in good activity against *E. coli* and *P. aeruginosa*, and uptake by the self-promoted uptake pathway (32). For the polycationic peptides, of which there are many (1), amidation of the COOH-terminal carboxyl is essential for the antimicrobial activity of certain peptides (34), whereas positive charge chain extension yields peptides with improved antibacterial activities (35,36). This has not yet been proved to be linked to self-promoted uptake, although self-promoted uptake has been implicated in the uptake of certain polycationic peptides.

The second method for enhancing antibiotic uptake, based on a knowledge of self-promoted uptake, is to use the ability of polycations to promote uptake of certain other molecules (including the β-lactam nitrocefin) (37). Of special note is the deacylated derivative of polymyxin B, termed polymyxin B nonapeptide (PMBN). Vaara and colleagues (38) have demonstrated that this compound can substantially enhance the anti–gram-negative bacterial activity of a range of antibiotics, especially hydrophobic ones that are normally excluded (2). Interestingly, the parent compound shows limited ability to enhance antibiotic activity. However, there is a simple explanation for these data. The PMBN is unable to form channels in artificial membranes and, presumably, in the cytoplasmic membrane of bacteria, in marked contrast to polymyxin B (39); consequently, PMBN has little antibiotic activity. Thus, it may be able to enhance the activity of other antibiotics because it can achieve a concentration sufficient to permeabilize the outer membrane, whereas at concentrations at which polymyxin B permeabilizes the outer membrane to other compounds, it self-promotes its own uptake, leading to killing. This would suggest that if the goal of such a compound was to enhance the uptake of other antibiotics, it should be designed so it interacts strongly with the outer membrane, but does not have intrinsically high antibacterial activity. As an example of such design limitations, we have synthesized by recombinant procedures a peptide, CEMA, that is a modification of a cationic *ce*cropin–*me*littin hybrid (CEME), in that it contains two extra positively charged amino acids at the COOH-terminus (36). This hybrid has a threefold higher affinity for LPS and superior permeabilizing ability, but decreases the MIC of co-added antibiotics only twofold whereas CEME is not synergistic with other antibiotics. Instead, the antibiotic with which the cationic peptide antimicrobials are maximally synergistic is polymyxin, presumably because they act synergistically at divalent cation-binding sites.

Other classes of compounds can disrupt outer membrane integrity and, together with the polycations just described, bear the group name "permeabilizers." In a survey study (37), it was demonstrated that organic monovalent cations and chelators, as well as ascorbate and acetylsalicylate, were capable of permeabilizing the outer membrane, although generally at much higher concentrations than those required for the better polycations.

4. *Other Uptake Routes*

It has been demonstrated that the gram-negative bacterial outer membrane is reasonably permeable to certain compounds for which there is no adequate description of an uptake route. Such compounds include certain steroids (40) and the fluoroquinolones, such as ciprofloxacin (41). For the latter antibiotics, uptake has been proposed to involve, in part, passage through porins in *E. coli*, although this may depend to some extent on the physiochemical character of the individual fluoroquinolone (42). For example, it has been proposed that fluoroquinolones utilize a novel non-

porin pathway in *P. aeruginosa* (43), whereas the limited influence of porin deficiency on *E. coli* susceptibility to fluoroquinolones (44) is also consistent with a nonporin pathway of uptake. This then may explain the excellent antimicrobial activity of the hybrid quinolone-β-lactams (44), which appear to be too bulky to pass rapidly through the channels of porins. This uptake mechanism bears some study, since it represents a hypothetical promiscuous-uptake system.

A better understood process of promiscuous uptake is passive diffusion across the cytoplasmic membrane (see Table 1). Any hydrophobic compound with significant lipid solubility (i.e., a suitably high partition coefficient) will partition into the cytoplasmic membrane (2). However, recently it has been convincingly argued (45) that fluoroquinolones and tetracyclines undergo a pH-dependent equilibrium between forms with different net charges and forms with zwitterionic or uncharged character. Furthermore, it has been suggested that only the uncharged form is membrane-permeable by passive diffusion. Despite the relatively low abundance of this uncharged form, it must be sufficient to permit a lethal concentration to accumulate in the cytoplasm, partly because of a higher pH in the cytoplasm that tends to shift the equilibrium toward the charged forms, which become trapped inside the cell.

B. Narrow Specificity Uptake Systems

1. Amino Acid Transport

A very wide variety of amino acid analogues exist (46). In certain cases, these have been demonstrated to be competitive inhibitors for uptake of the amino acid they resemble (9,47). Their actual mode of action often depends on the translational synthesis of inactive proteins. Some of these amino acids are toxic, presumably because they are transported also by mammalian cells. The best-studied bacteria-selective amino acid analogue is cycloserine, which has some useful antituberculosis activity. Cycloserine is specifically transported by the high-affinity, energized D,L-alanine transport system of *Streptococcus faecalis* and the D-alanine transport system of *E. coli* (9). This antibiotic works by inhibiting D-alanine racemase and D-alanine-D-alanine synthase, two enzymes involved in peptidoglycan side chain biosynthesis. Other antibiotics that are amino acid analogues include hadacin (an L-aspartate analogue) and azaserine and diazo-oxonorleucine (DON) (glutamine analogue). However, to my knowledge, their transport has not been studied.

Recently it has been demonstrated that *P. aeruginosa* (48) synthesizes an outer membrane protein, OprD (also known as D2), that enhances uptake of the β-lactam imipenem and related zwitterionic carbapenems.

OprD forms channels across the outer membrane, with a binding site for basic amino acids and zwitterionic carbapenems. This probably reflects the fact that although these carbapenems, like other β-lactams, are dipeptide analogues, they uniquely resemble the preferred dipeptide substrate of *oprD*, since apparently, no other β-lactams can use this channel (48,49).

2. Other Metabolites

Phosphomycin, a peptidoglycan biosynthesis inhibitor, is an α-glycerophosphate analogue, reported to access the cytoplasmic membrane uptake system for this compound (9). Its ability to access the hexose-6-phosphate permease is, however, somewhat more difficult to understand. Arsenate, an antimicrobial substance that is also a general metabolic poison and phosphate analogue, can use the phosphate uptake pathways of the cytoplasmic membrane (50) and outer membrane (51). Although not studied, one can assume that nucleotide analogues with antibacterial activity, such as psicofuranine, decoyinine, the hydroxyphenyl-azidopyrimidines—iododeoxyuridine, arabinosylcytosine, and arabinosyladenosine (9)—use normal nucleotide uptake pathways across the cytoplasmic membrane. In addition, the antibiotic albicidin utilizes the Tsx protein, a nucleotide-specific channel, to cross the *E. coli* outer membrane (52).

III. TRANSPORT AS A TARGET

There are no instances known to me in which an antimicrobial agent acts exclusively by inhibiting bacterial transport. Thus, the following represents a general discussion of inhibitory compounds, both known and potential.

A. Inhibition of Bacterial Energization

Bacteria generate energy for various cellular functions in one or both of the following ways: through substrate level phosphorylation (fermentation), leading to ATP production; or by generation of a proton gradient across the cytoplasmic membrane, oriented internally negative and alkaline relative to the outside (53). The latter, called the proton motive force, involves protons pumped across the cytoplasmic membrane by the cytoplasmic membrane-bound electron transport chain, or the movement of protons through membrane-bound ATPase as a result of ATP hydrolysis. One important use of the energy generated by these processes is for active transport of compounds from the extracellular milieu. Thus, any inhibitor of cellular energization has the potential, by definition, to be an inhibitor of transport, although transport is by no means the only process inhibited.

Active transport systems can be broadly divided into those energized directly by ATP and requiring a specific periplasmic binding protein [so-called (osmotic) shock-sensitive systems]; those (shock-resistant systems) energized by the proton motive force (or by one or other of the components of it—namely, the electrical potential gradient or the pH gradient); and those energized by direct phosphorylation, using the process of group translocation (8). Those systems that require high-energy phosphates (shock-sensitive system or group translocation) are inhibited by the phosphate analogue arsenate, which inhibits ATP formation through substrate-level phosphorylation or by dicyclohexylcarbodiimide (DCCD), which inhibits ATP synthesis directed by the proton motive force, mediated by the Na^+, K^+-ATPase. The shock-resistant systems are inhibited by ionophores, which shuffle monovalent cations or protons across the cytoplasmic membrane to neutralize the proton motive force. Such ionophores (9,54) include the known antibiotics, valinomycin, nonactin, monensin, and nigericin; the channel-forming antibiotic gramicidin A acts similarly. In addition, under conditions in which cells are energized through electron transport (i.e., respiration), electron transport inhibitors (e.g., KCN) block transport. Nevertheless, all of the foregoing agents tend to be quite toxic, owing to their effects on mammalian energy generation. Therefore, with the exception of monensin, which has been used as a feed additive for chickens, they are now used only as biochemical tools for studying energetics.

B. Inhibiting Uptake of Essential Metabolites

For metabolite transport to be considered a target, the transport system must be obligately required for bacterial growth. Given the multitude of transport systems for carbon sources and the intrinsic ability of most bacteria to synthesize all amino acids and nucleotides, this is a substantial constraint. There are, however, selected bacteria that are amino acid auxotrophs. For example, the multiply antibiotic-resistant bacterium *Xanthomonas maltophilia* is a natural methionine auxotroph, whereas the obligate intracellular pathogen *Chlamydia trachomatis* is unable to synthesize cysteine, histidine, or ATP. Thus, these significant pathogens are potential targets for amino acid or ATP analogues that inhibit uptake of these required amino acids or ATP. Phosphate and sulfate are also required by bacteria, and known transport inhibitors include arsenate (50), for phosphate, and thiosulfate and vanadate (55), for sulfate. However, it is likely that such agents would also inhibit mammalian cell transport, making them potentially toxic.

Another essential metabolite is iron. Iron uptake was previously discussed in detail (see Sec. II.A.1). Despite the substantial heterogeneity of iron uptake systems, two potential classes of inhibitors could be envisaged. One class would include inhibitors of the function of TonB, the central player in energization of siderophore–iron uptake. A second class would comprise inhibitors of the binding of transferrin–iron or lactoferrin–iron complexes to their specific outer membrane receptors in bacteria (including several important pathogens) that transport iron by this route. No such inhibitors have yet been reported. A third possible site of intervention is in the global regulation of iron transport that is mediated through a central aporepressor, Fur.

C. Channel-Forming Compounds

Although not, strictly speaking, transport inhibitors, the channel-forming compounds destroy cytoplasmic membrane integrity and, thereby, prevent transport and encourage leakage of internal cell constituents. Such compounds include the related cationic antibiotics polymyxin B and colistin (39), the cationic antiseptic chlorhexidine (9), the cyclic decapeptides gramicidin S and the tyrocidins (9), and the antimicrobial cationic peptides magainins, cecropins, defensins, and others (1). Several of these are used medicinally or are being considered for commercial application; for example, the magainin (MSI-78) is currently in Phase III clinical trials.

D. Secretion Mechanisms

There are two general classes of secretion systems in bacteria, both of which involve the passage of molecules across bacterial membranes. One class involves export of molecules to cell compartments beyond the cytoplasmic membrane. The second involves excretion of molecules into the environment of the bacterium. The former involves export of proteins (56) by a conserved system involving a cytoplasmic membrane apparatus and an NH_2-terminal leader sequence on the exported protein, as well as export of lipopolysaccharides, peptidoglycan precursors, and other carbohydrate-containing molecules. These would appear to be potential targets for antimicrobials and, indeed, the antibiotics enduracidin A, vancomycin, monomycin, and tunicamycin, all cause accumulation of membrane-bound undecaprenol lipid intermediates required in the biosynthesis and export of peptidoglycan (and lipopolysaccharide O-antigen) precursors (9).

The second class involves excretion of proteins involved in the pathogenesis of certain bacteria, including extracellular proteases, lipases, he-

molysins, and toxins. The generally high level of conservation of these excretion systems, especially the general secretory pathway and the hemolysin-like secretory pathway (56), seems to offer opportunities for antimicrobial intervention, possibly leading to decreased pathogenic potential, rather than bacterial death or stasis. Cerulenin, an inhibitor of fatty acid synthase, exhibits such activity (57).

E. ATP-Requiring Transport Systems

Bacterial shock-sensitive transport systems contain a peripheral membrane protein, with a conserved motif that is involved in ATP binding and energization of transport (47). This motif is shared by two highly important mammalian cells for drug intervention; namely, the multidrug resistance (MDR) protein and the cystic fibrosis transmembrane regulator (CFTR) proteins (58). Given that pharmaceutical companies are directing considerable effort toward finding inhibitors for the MDR protein, this may offer a potential source of compounds active against the homologous bacterial proteins involved in energization of shock-sensitive transport systems.

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11

Internalization of Amphotericin B and Other Polyene Antifungals in Mammalian Cells: A Possible Origin of Their Toxicity

Jacques Bolard and Aline Vertut-Doï
Université Pierre et Marie Curie, Paris, France

I. INTRODUCTION

Amphotericin B (AmB) and nystatin (Fig. 1) are the most widely used drugs in the treatment of systemic fungal infections. Other polyenes, such as mepartricin or hamycin, are less frequently used, as their higher toxicity limits their usefulness, despite their higher activity. The polyene antibiotics were discovered 40 years ago and, therefore, could be considered old drugs, not deserving further attention. Actually, this is not true for the following reasons:

1. Systemic fungal diseases occur primarily in individuals with defective immune response and thus are becoming prevalent as the population of immunosuppressed, acquired immunodeficiency syndrome (AIDS), cancer, and transplant patients increases. For example, a recent study has shown that AmB use increased almost tenfold between 1978 and 1988 at Duke University Medical Center (1).
2. The clinically used formulation of AmB, Fungizone, has several serious side effects, especially severe nephrotoxicity. Therefore, it would be highly desirable to design new formulations with decreased host toxicity. Indeed, new derivatives of AmB are currently in clinical trials, as are new delivery systems, particularly liposomes. One liposomal formulation is already commercially available.