Eur Biophys J (2011) 40:387–397
DOI 10.1007/s00249-011-0682-7

REVIEW

Antimicrobial peptides with cell-penetrating peptide properties and vice versa

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Received: 6 October 2010 / Revised: 2 February 2011 / Accepted: 3 February 2011 / Published online: 19 February 2011

Abstract Antimicrobial peptides (AMPs) are a group of peptides that are active against a diverse spectrum of microorganisms. Due to their mode of action, AMPs are a promising class of molecules that could overcome the problems of increasing resistance of bacteria to conventional antibiotics. Furthermore, AMPs are strongly membrane-active and some are able to translocate into cells without the necessity for permanent membrane permeabilization. This feature has brought them into focus for use as transport vectors in the context of drug delivery. Since the plasma membrane restricts transport of bioactive substances into cells, great research interest lies in the development of innovative ways to overcome this barrier and to increase bioavailability. In this context, peptide-based transport systems, such as cell-penetrating peptides (CPPs), have come into focus, and their efficiency has been demonstrated in many different applications. However, more recently, also some AMPs have been used as efficient vectors for intracellular translocation of various active molecules. This review summarizes recent efforts in this interesting field of drug delivery. Moreover, some examples of the application of CPPs as efficient antimicrobial substances will be discussed.

Membrane-active peptides: 455th WE-Heraeus-Seminar and AMP 2010 Workshop.

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Keywords Antimicrobial peptides · Drug delivery · Cell-penetrating peptides · Cellular uptake mechanism

Introduction

All multicellular organisms have developed some kind of defense system, based on either molecular or cellular components. For immediate defense, the innate immune system provides protection against infection from other organisms and is found in all plants and animals (Boman 2000). These nonspecific defense mechanisms occasionally include the release of effector molecules such as antimicrobial peptides (AMPs). AMPs are antibiotic molecules that are active against a broad spectrum of microorganisms (Brogden 2005; Koczulla and Bals 2003). Microbial resistance to conventional antibiotics and the unique mode of action of antimicrobial peptides have brought them into the focus of research and made these peptides promising candidates for the development of a new class of antibiotics. All AMPs share the ability to interact strongly with cell membranes, some without the necessity for permanent membrane permeabilization. In this way they are able to overcome the plasma membrane and translocate into the cell interior. This feature is of great interest, since the plasma membrane restricts uptake of large, hydrophilic or charged molecules. Therefore, a lot of interesting new drug candidates fail to undergo further development as cellular drugs due to their poor membrane-crossing abilities. Attachment to membrane-active peptides that act as delivery vectors is one approach to solve the problems of efficient cellular uptake. Here, so-called cell-penetrating peptides (CPPs) have been successfully used since their discovery 20 years ago. CPPs are a family of peptides that are structurally diverse but share the ability to translocate a

wide range of different bioactive molecules into living cells (Järver et al. 2010).

Due to the strong membrane association of AMPs it is not unexpected that they are able to translocate into the cytoplasm, a fact that makes AMPs also an interesting vector strategy as drug transporters. This review will summarize recent efforts in the use of some antimicrobial peptides as drug delivery vectors. Furthermore, CPPs exhibiting antimicrobial properties will be described. Examples of different peptides that are usually used as AMPs as well as CPPs are given in Table 1.

Antimicrobial peptides

AMPs were first discovered in bacteria some decades ago and later also in plant, insect, and amphibian venoms and tissue extracts. Until now, numerous antimicrobial peptides have been isolated from natural sources. Additionally, a lot of antimicrobial structures have been de novo designed and synthetically produced. An online database of hundreds of reported AMPs can be found at http://www.bbcm.univ.trieste.it/~tossi/amsdb.html or at http://aps.unmc.edu/AP/main.php. Basically, AMPs have the ability to kill pathogenic microorganisms, including Gram-positive and Gram-negative bacteria, viruses, protozoa, and fungi. Moreover, they play an important role in the innate immune system of higher organisms such as plants, insects, amphibians, and mammals (Lehrer and Ganz 1999; Tossi et al. 2000). Higher organisms produce them on epithelial surfaces or directly in endothelial and phagocytic cells, thus exhibiting a defense system for prevention of colonization and infections.

AMPs generally consist of fewer than 60 amino acid residues, which are typically ordinary L-amino acids. Also they have a positive net charge, normally in the range of

Table 1 Examples of commonly used CPPs and AMPs and some of their properties related to interaction with membranes

Name	Sequence	Structure	Proposed mechanisms[c]	References
AMPs				
Pyrrhocoricin	VDKGSYLPRPTPPRPIYNRN[b]	Random coil/reverse turns at the termini	Receptor-mediated	Otvos (2000)
Lactoferricin (**hLF peptide**)	VSQPEAT**KCFQWQRNMRKVRGPPVSCIKR** DSPIQI[b] | GRRRRSVQWCA	β-Turn/loop	Direct penetration/ pore formation	Duchardt et al. (2009)
Bac7 **(Bac7 1–24)**	**RRIRPRPPRLPRPRPRPLPFPRPG**PRPIPRPL PFPRPGPRPIPRPLPFPRPGPRPIPRP	Hybrid of PPII helix and α-helix	Receptor-mediated/ pore formation	Sadler et al. (2002)
PG-1 (SynB1)	RGGRLCYCRRRFCVCVGR (RGGRLSYSRRRFSTSTGR)	Cationic, amphipathic antiparallel β-sheet	Endocytosis/ pore formation	Rousselle et al. (2000) Kokryakov et al. (1993)
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES[b]	α-Helical	Pore formation	Oren et al. (1999)
Buforin II (BF2d)	TRSSRAGLQFPVGRVIIRLLRK TRSSRAGLQWPVGRVIIRLLRKGGC	Extended amphipathic, α-helical[a]	Direct penetration	Park et al. (1998)
CPPs				
sC18	GLRKRLRKFRNKIKEK[b]	Amphipathic, α-helical	Endocytosis	Neundorf et al. (2009)
Tat (48–60)	GRKKRRQRRRPPQ[b]	Random coil/PPII helix[a]	Direct penetration/ pore formation	Vives et al. (1997)
Penetratin	RQIKIWFQNRRMKWKK[b]	Amphipathic, α-helical[a]/ β-sheet (higher conc.)	Direct penetration/ endocytosis	Derossi et al. (1994)
pVEC	LLIILRRRIRKQAHAHSK[b]	Amphipathic, β-sheet[a]	Direct penetration/ transporter-mediated	Elmquist et al. (2001)
Pep-1	KETWWETWWTEWSQPKKKRKV[b]	Amphipathic, α-helical[a]	Direct penetration/ pore formation	Morris et al. (1997)
Transportan (**TP10**)	GWTLNS**AGYLLGKINLKALAALAKKIL**[b]	Amphipathic, α-helical	Endocytosis/ direct penetration	Pooga et al. (1998)
MAP	KLALKLALKALKAALKLA[b]	Amphipathic, helical	Multiple mechanisms	Steiner et al. (1991)

[a] Present at high lipid-to-peptide ratio conditions; [b]peptides are *C*-terminally amidated; [c]mode of action is described differently regarding concentration, cell type, and/or conditions

+4 to +6, which is due to the frequently present lysines and arginines in the amino acid sequence (Hancock 1997). Additionally, they often consist of nearly 50% hydrophobic residues. Therefore, these peptides often exhibit spatially separated hydrophobic and charged regions and show amphipathic properties (Powers and Hancock 2003), a feature that makes them usually membrane active. Despite those similarities, their structure is highly diverse, and the peptides vary considerably in length, amino acid sequence, and secondary structure. For this reason, there exist various ways of classification. For instance, they can be classified into two groups concerning their toxicity to microorganisms and to mammalian cells. Here, peptides that are active against different types of bacteria such as cecropins (Steiner et al. 1981) are related to the first group, being active against microorganisms but not against mammalian cells. Also included are peptides that are active against Gram-positive and Gram-negative bacteria, such as magainins or some cathelicidins (Lehrer and Ganz 2002). AMPs that are toxic to both microorganisms and mammalian cells include, for example, melittin or LL-37 (Shai 2002) and belong to the second group in this classification. Considering their secondary structure, AMPs can be divided into four different groups (Jenssen et al. 2006), the first being linear, α-helical peptides without the presence of cysteines in the sequence, such as melittin (Steiner et al. 1981), and second, peptides with β-sheet structures that contain two or more disulfide bridges in their peptide structures, such as the protegrins (Steinberg et al. 1997). AMPs with intermolecular disulfide bonds exhibiting loop/hairpin-like structures, such as bactenecin (Romeo et al. 1988), belong to the third group. The final group are peptides with predominance of one or more distinct amino acids, such as the proline/arginine-rich peptide Bac7 (Gennaro et al. 1989). Other ways to classify AMPs include grouping them into families that are based on conserved sequence segments (e.g., defensins or cecropins) or to sort them based on their natural source (e.g., neutrophils).

AMPs display antimicrobial activity at micromolar concentrations or below. Compared with conventional antibiotics, the mechanism by which they kill bacteria is very rapid, which means within minutes in vitro. In contrast, conventional antibiotics such as norfloxacin or vancomycin need at least 4 h or even 24 h to kill bacteria (Steinberg et al. 1997). In addition, besides membrane disruption, their bactericidal mode of action involves interference with metabolic processes or targeting of cytoplasmic components (Brogden 2005; Kragol et al. 2001b). Predominantly, they act by disrupting the integrity of cell membranes through interaction of their cationic domains with negatively charged cell surface components, mostly phospholipids. This is based on the architectural and biochemical composition of the cellular membrane. It is also known that these peptides can not only act directly as microbe killers but also play an important role in tissue processes. The defensins, for example, are described to be involved in various signaling events, such as wound repair, cell migration or chemotaxis (Lehrer 2004).

Another specific feature of these peptides is their broad spectrum of activity against various species such as Gram-positive and Gram-negative bacteria, fungi, parasites, enveloped viruses, and even tumor cells (Hancock 2001; Koczulla and Bals 2003; Otvos 2005; Powers and Hancock 2003). All this has brought AMPs into the focus as potential candidates for a new generation of antibiotics.

However, also direct membrane translocation has been shown to occur for many of them (Matsuzaki et al. 1995; Zhang et al. 2001), an observation that may not be surprising considering their ability to interact strongly with cell membranes. Thus, different AMP structures have also been used as templates to design new and efficient delivery vectors acting like cell-penetrating peptides (Fischer et al. 2005).

Cell-penetrating peptides

The observation that some intracellular proteins are able to pass through the cell membrane was first made for the Tat and pAntp proteins (Derossi et al. 1994; Frankel and Pabo 1988). Since then, many other proteins that contain peptide domains responsible for membrane-translocating properties have been identified.

Like AMPs, they carry a positive net charge that is due to a large amount of basic amino acids (such as arginine and lysine) in the peptide structure, however some of them are characterized by an amphipathic structure, too. The number of CPPs developed so far is growing, all of them being derived from different sources that can be divided into natural, synthetic or chimeric (Lindgren et al. 2000). Table 1 includes some frequently used CPPs.

The mechanism of cellular uptake of CPPs is still not completely understood and is controversially discussed. Both endocytotic and nonendocytotic routes have been suggested and observed (Jiao et al. 2009; Vives et al. 2008). Moreover, it has been speculated that often more than one uptake pathway is possible, dependent on factors such as the peptide concentration applied, the cell line used, the cargo attached, and the overall incubation conditions (Jiao et al. 2009; Walther et al. 2009; Tünnemann et al. 2006).

Furthermore, CPPs are mostly low in toxicity. Until now, a wide range of different applications for translocation of macromolecules into living cells has been described, including transport of proteins (Räägel et al. 2009),

oligonucleotides (Said Hassane et al. 2010), quantum dots (Walther et al. 2008), polysaccharides (Henriques et al. 2005), nanoparticles (Lewin et al. 2000; Zhao et al. 2002), chemotherapeutics (Dubikovskaya et al. 2008; Lindgren et al. 2006), polymers (Nori et al. 2003), and liposomes (Torchilin et al. 2001).

Mechanisms of internalization

Several different mechanisms of interaction with cell membranes are described for CPPs as well as for AMPs, and both groups share some of them. Common to both groups are interactions of cell membrane components with the charged amino acid residues of the peptides. This is probably the first step in cell association that leads to cellular uptake. Anionic phospholipids or phosphate groups of lipopolysaccharides (for Gram-negative bacteria) or acidic polysaccharides, teichoic acids, and lipoteichoic acids (for Gram-positive bacteria) are the membrane components at the cell surface responsible for generating an overall negative net charge. Here, the binding of positively charged or amphipathic peptides is possible (Tossi et al. 2000). Furthermore the lipid bilayer of bacterial membrane contains mainly lipids with negatively charged phospholipid headgroups. In contrast, fungi cells exhibit a zwitterionic lipid bilayer composition, and here, the uptake is driven by a hydrophobic *N*- or *C*-terminus or particular amino acids of the peptide sequence resulting in accumulation of the peptide, which finally induces strong hydrophobic binding to the membrane. In mammalian cells, which also exhibit a zwitterionic lipid bilayer composition, the interaction with positively charged amino acid residues of the peptide proceeds presumably via the negatively charged residues of the cell-surface proteoglycans or glycosaminoglycans (e.g., heparin sulfate and/or heparin) (Console et al. 2003). Sterols such as cholesterol and ergosterol are generally neutral and can be found in eukaryotic but rarely in prokaryotic cell membranes. The presence of such sterols in membranes may reduce the uptake or membranolytic activity of AMPs by altering the membrane fluidity and thereby stabilizing the lipid bilayer and interfering with the membrane insertion of the peptides (Matsuzaki 1999). In fact, direct electrostatic interaction between positively charged residues of the peptide and negatively charged membrane components is required for internalization, regardless of the eventual mechanism of cellular uptake.

For the subsequent cellular uptake pathway of CPPs, different routes have been proposed: on the one hand, energy-dependent routes such as internalization via different endocytotic pathways, where routes such as caveolae-mediated and clathrin-mediated endocytosis as well as macropinocytosis are discussed (El-Andaloussi et al. 2005; Nakase et al. 2004; Richard et al. 2003; Vives et al. 2003); on the other hand, also energy-independent pathways may play a role, leading to endocytosis, direct translocation, and cell penetration, whereby formation of hydrophilic pores through which the peptides can enter the cytoplasm may occur (Deshayes et al. 2004; Simeoni et al. 2003). Due to the short opening and closing rate of such pores, remaining microseconds or less, no membrane leakage that would lead to cell lysis is observed. Alternatively, local destabilization of the cellular membrane may appear, leading to peptide internalization. The inverted micelle model describes a case where interaction of the peptide with the cell membrane leads to perturbance of the lipid bilayer, resulting in the formation of inverted micelles. Further interaction of the peptides with membrane components would lead to an inverse process, resulting in destabilization of the inverted micelles and release of the peptides into the cell (Derossi et al. 1996). Although the major route is described to be endocytotic, strong evidence for minor routes such as direct translocation across the membrane bilayer has been found (Järver and Langel 2006). Often, a mixture of all mechanisms seems to occur depending on the investigated peptide, cell line, cargo, and experimental conditions such as concentration or temperature (Tünnemann et al. 2006; Jiao et al. 2009).

However, for AMPs, two different mechanisms of action have been described, classified as a pore-dependent and a pore-independent mechanism, where the first is the most common. A model that characterizes the activity of many antimicrobial peptides is the Shai–Matsuzaki–Huang model (SMH) (Matsuzaki 1999) in which the peptide first interacts with the membrane, followed by displacement of lipids leading to alteration of the membrane structure, and finally, in some cases, to entry of the peptide into the cell. Thus, most AMPs kill their microbial target by membrane damage or permeabilization, predominantly acting via disruption of cell membrane integrity through interaction with the phospholipid molecules (Jelinek and Kolusheva 2005). Above a certain concentration, AMPs perturb membranes by formation of pores that have a lifetime in the millisecond range. Different models of pore formation mechanisms include the barrel-stave and toroidal (wormhole) models (Brogden 2005). According to the barrel-stave model, the peptide helices form a cluster in the membrane with a central cavity. Here, the hydrophobic peptide region is oriented in the same direction as the lipid core region, and the hydrophilic region is within the center of the pore. The second model discussed is the toroidal pore model, where the peptide helices insert into the membrane by bending the lipid layer such that peptides and lipid head groups line the pore. Peptides that are too short to span the bilayer often act via the carpet model (Fernandez et al. 2009). In the carpet-like model, also

called the detergent-like mechanism, the peptides accumulate parallel to the cell surface, forming a layer or carpet, followed by displacement of lipids, alteration of the membrane structure, and in certain cases entry of the peptide into the cell. At higher concentrations, this finally results in disruption of the membrane and consequently cell lysis. Another proposed pore-formation mechanism is molecular electroporation, where the bilayer structure is locally disrupted through a transmembrane electric field that is caused by binding of the highly charged peptide to the membrane (Binder and Lindblom 2003). In addition, a mechanism termed the "leaky slit" was proposed, where the peptides are oriented perpendicular to the membrane and aggregate side by side to form an amphipathic ribbon. Then, the other side of the membrane bends onto itself, forming a slit (Zhao et al. 2006). Alternatively, the "sinking raft" model describes a process whereby the peptides form aggregates which can diffuse through the membrane (Pokorny et al. 2002).

As described before, the membrane activity of AMPs is probably dependent on the lipid bilayer composition of the organisms (Huang 2000). Thus, for a small number of AMPs, it is suggested that they enter cells by direct penetration without destroying the membrane [e.g. buforin II (Park et al. 1998)], or, as in eukaryotic cells, by endocytosis [e.g., Bac7(1–35) or LL-37 (Lau et al. 2005; Tomasinsig et al. 2006)], aggregate formation (inverted micelle) [e.g., polyphemusin (Powers et al. 2005)] or transporter-mediated internalization mechanisms [e.g., apidaecin (Casteels et al. 1993)]. After uptake into the cytoplasm, they often interact with several intracellular targets such as DNA and RNA and affect metabolic functions, finally leading to cell death (Brogden 2005). Other modes of action are, for example, inhibition of cell wall synthesis, nucleic acid or protein synthesis, or inhibition of the activity of different enzymes.

AMPs: application as drug delivery vectors

A lot of different delivery vectors and techniques have been developed to transport therapeutic substances across the cell membrane and to their target site. Applications vary from use of viral vectors (Anderson 1998) or peptide ligands that internalize via receptor-mediated endocytosis (Langer et al. 2001) to physical methods such as microinjection or electroporation (Wong and Neumann 1982). Also physicochemical approaches such as liposome encapsulation (Luo and Saltzman 2000) are commonly used. However, these methods suffer from various drawbacks such as inefficient drug delivery, cellular damage, toxicity, limited applicability in vivo, and restrictions due to the properties of the drug and the cell type. In this respect, use of membrane-permeable peptides has attracted research interest.

As mentioned before, AMPs are described to show CPP properties as well. Therefore, they can be used as delivery vectors for several therapeutic and diagnostic molecules in treatment of cancer and genetic, cardiovascular, inflammatory, and infectious diseases. Several representatives will be discussed in the next section. In Table 2, examples of transported cargos of antimicrobial peptides are summarized.

Pyrrhocoricin, for example, consists of 20 amino acids and is an antimicrobial peptide isolated from insects, being effective against Gram-negative bacteria but almost inactive against Gram-positive strains (Otvos 2000). Also for some pyrrhocoricin analogs a wide activity spectrum has been described (Otvos et al. 2000). These peptides are able to bind and deactivate their bacterial target protein DnaK stereospecifically without disrupting the membrane integrity (Kragol et al. 2001a). Furthermore, pyrrhocoricin itself has been used as a drug delivery system. In their study, Otvos et al. investigated a modified pyrrhocoricin dimer that could successfully deliver peptide antigens (NPK^d epitope) into dendritic cells and human fibroblasts (Otvos et al. 2004).

Another example is the antimicrobial protein lactoferrin (hLF), the human milk protein, which is a very important protein in immune defense due to its antifungal, antimicrobial, and antiviral activities (González-Chávez et al. 2009). Normally it is cleaved in the gastrointestinal tract through pepsin digestion to a shorter peptide, named lactoferricin.

Table 2 Various cargos delivered by selected antimicrobial peptides

Peptide	Cargo	Cell type	References
Pyrrhocoricin	Antigen (NPK^d peptide)	Dendritic cells	Otvos et al. (2004)
Lactoferricin (hLF peptide)	Protein (Alexa 488-labeled streptavidin)	HeLa	Duchardt et al. (2009)
Bac7 (Bac7 1–24)	Protein (NeutrAvidin)	Murine monocytes	Sadler et al. (2002)
SynB1/PG-1	Doxorubicin	Rat brain	Rousselle et al. (2000)
sC18	Organometallic complexes	MCF-7, HT-29	Splith et al. (2010a)
LL-37	Oligonucleotides/DNA-plasmid	COS-7, CHO-K1	Zhang et al. (2010), Sandgren et al. (2004)
Buforin II (BF2d)	Protein (GFP)	HeLa	Takeshima et al. (2003)

No toxicity of the peptides was observed at the conditions used

This truncated version consists of 49 amino acids (amino acids 20–68 of the parent sequence) and exhibits antimicrobial, antiviral, antitumor, and immunological activity (Gifford et al. 2005). Another shortened peptide sequence, named hLF peptide, which consists of the amino acids 38–59 (19–40 of lactoferricin), was described to enter different cells efficiently (e.g., HeLa or rat IEC-6). Previously, a sequence comprising amino acids 37–59 (18–40 of lactoferricin) was also found to possess antimicrobial properties, even when the cysteine residues were covalently blocked (Bellamy et al. 1992). The hLF peptide contains two cysteines forming a cyclic peptide through a disulfide bridge, and its sequence overlaps with the lipopolysaccharide-binding region of lactoferricin. The uptake mechanism of the hLF peptide seems to be concentration dependent, and for concentration higher than 10 μM, rapid delivery into the cytoplasm and nucleus is observed. Furthermore, the uptake pathway was determined to be sensitive to rottlerin, a protein kinase inhibitor with specificity for protein kinase C (PKC). This was also observed for the uptake of arginine-rich CPPs such as Tat and R9 (Duchardt et al. 2007). The uptake efficiency is supposed to be conformation dependent, because the cyclic structure is required for binding to heparin sulfate and correlates with lipid-induced conformational changes. Several examples of efficient cargo delivery have been described for the hLF peptide; especially proteins and high-molecular-weight complexes have been successfully transported (Duchardt et al. 2009).

Another peptide which combines cell-permeation and antimicrobial properties is Bactenecin 7 (Bac7) (Skerlavaj et al. 1990; Tani et al. 1995). This is a linear 59-residue protein that was isolated from large granules of bovine neutrophils. Bac7 belongs to the bactenecin family and consists of four 14-residue repeats. It also belongs to the Pro/Arg-rich family and was described to be antimicrobially active against Gram-negative bacteria in a micromolar range but not against Gram-positive strains (Frank et al. 1990; Gennaro et al. 1989). The antimicrobial effect is caused by inhibition of the intracellular protein synthesis machinery in a two-step mechanism, where the first is entry of the peptide into the cytoplasm and the second is intracellular inhibition of its target (Skerlavaj et al. 1990; Tani et al. 1995). Interestingly, the *N*-terminal region is responsible for both the antimicrobial and the cell-penetrating properties. Therefore, fragments of this region have been used as templates for antimicrobial and delivery peptides, one example being the fragment Bac1-24. Generally, longer segments of Bac-7, containing antibacterial and intracellular delivery regions, have antibacterial and cell-permeating activity. In contrast, shorter fragments with a hydrophobic or cationic region show only cell-penetrating and no antimicrobial properties, suggesting that the mechanism of action and the crucial amino acids are different (Sadler et al. 2002).

Also, SynB vectors from the antimicrobial peptide protegrin-1 (PG-1) can be used for cargo delivery purposes. Protregins are small cysteine- and arginine-rich antimicrobial peptides. PG-1 consists of 18 amino acids and was originally derived from porcine leukocytes (Kokryakov et al. 1993). It shows potent activity against fungi, bacteria, and several enveloped viruses. Like other AMPs, it interacts with the lipid matrix of bacterial membranes and forms pores (Mangoni et al. 1996). This uptake is related to voltage-dependent and ion-selective pore formation in the lipid matrix of bacterial membranes (Sokolov et al. 1999). Furthermore, as for the above-described hLF peptide, the internalization is dependent on its cyclic structure. PG-1 possesses two disulfide bridges and an amphipathic structure in which the positively charged and hydrophobic residues are separated. The replacement of four cysteines and two valines of PG-1 led to linear peptides (SynB1) still able to penetrate cells efficiently but without being cytolytic to them. With the help of this, peptide transport of covalently coupled doxorubicin to the brain has been reported. The blood–brain barrier was crossed with high efficiency and without any compromise to its integrity (Rousselle et al. 2000, 2001).

Another example is buforin II, a 21-amino-acid antimicrobial peptide that was discovered in stomach tissue of Asian toad. It penetrates through the cell membrane without destroying it and kills bacteria by binding to nucleic acids. BF2d, which is a modified analogue of buforin II, exhibits cell-penetrating properties and is able to deliver the GFP protein to HeLa cells (Takeshima et al. 2003).

Recently, we have developed a cathelicidin-derived carrier peptide, sC18. This peptide originates from the 18-kDa cationic antimicrobial protein (CAP18) that was first isolated from rabbit leukocytes. Like the hLF protein, CAP18 is a lipopolysaccharide binding protein with an α-helical structure (Chen et al. 1995). CAP18 itself and also shortened variants exhibit antimicrobial properties in the lower micromolar range (Larrick et al. 1993). Residues 106–125 of the *C*-terminal region of CAP18 were identified as highly cationic, exhibiting an amphipathic α-helical conformation possibly responsible for the antimicrobial activity (Tossi et al. 1994). Recently, we investigated the cell-penetrating properties of another shortened form from the same region, consisting of amino acids 106–121 of CAP18 (Neundorf et al. 2009). This so-called sC18 peptide turned out to exhibit promising internalization abilities into several different cell lines without being toxic in the tested concentration range. Furthermore, also antimicrobial activity in the micromolar range against *Escherichia coli* and *Micrococcus luteus* has been observed (unpublished results). In recent studies we used this peptide successfully as a transporter for cytostatic organometallic complexes (Neundorf et al. 2008; Splith et al. 2010b; Splith et al. 2010a).

The LL-37 peptide was the first identified α-helical AMP from a human source. It also belongs to the cathelicidin family, with the human cationic antimicrobial peptide (CAP18) as its precursor. LL-37 consists of 37 amino acids and exhibits a net charge of +6 at neutral pH (Oren et al. 1999). Furthermore, it has a broad antimicrobial spectrum and shows high activity [minimum inhibitory concentration (MIC) < 10 μg/ml] against both Gram-positive and Gram-negative bacteria such as *Pseudomonas aeruginosa*, *E. coli*, *Listeria monocytogenes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (Turner et al. 1998). Interestingly, LL-37 also exhibits cytotoxic activity against eukaryotic cells by inducing membrane disruption via a carpet-like mechanism (Henzler Wildman et al. 2003; Oren et al. 1999). The drug delivery potential was proven by the delivery of non-covalently linked fluorescently labeled oligonucleotides (Zhang et al. 2010) as well as by the delivery of plasmid DNA into different cell lines (Sandgren et al. 2004).

CPPs with AMP properties

Also for the group of membrane-active cell-penetrating peptides, antimicrobial properties have been observed. Some examples of common and well-known CPPs that are also used as efficient AMPs will be briefly discussed in this section. In Table 3, the reported antimicrobial activities of the different cell-penetrating peptides against Gram-positive bacteria, Gram-negative bacteria, and fungi are summarized.

In recent years it has been discovered that the Tat peptide shows potent antibacterial activity (MIC 2–8 μM) against a broad spectrum of pathogens including Gram-positive and Gram-negative bacteria such as *S. aureus* and also fungi such as *Saccharomyces cerevisiae* and *Candida albicans* (Jung et al. 2006; Jung et al. 2008; Zhu and Shin 2009b). The peptide internalizes without any damage to the cell membrane, thus being cytotoxic inside the cells, leading for example to fast accumulation in the nucleus in fungi, where it causes cell cycle arrest in G1 phase. The uptake of the Tat peptide seems to be sequence dependent and not induced by its secondary structure (Vives et al. 1997).

Nearly 20 years after its discovery, it was observed that the CPP penetratin is a potent antimicrobic against Gram-negative and Gram-positive bacteria such as *Bacillus megaterium*. An MIC of 0.5–4 μM was measured, and the peptide showed no cytotoxicity against mammalian cells. For its mechanism it was assumed that penetratin targets the intracellular components of microorganisms by penetrating lipid bilayers through potential- or energy-dependent pathways (Palm et al. 2006; Zhu and Shin 2009a).

The 18-amino-acid peptide pVEC is derived from the vascular endothelial-cadherin protein and consists of 13 cytosolic and 5 transmembrane amino acids of the parent sequence. Uptake takes place by a nonendocytotic mechanism of translocation without alteration of plasma membrane permeability or cell morphology (Elmquist et al. 2001). After the internalization process, it is mainly localized in nuclear structures and was used as a carrier for peptide nucleic acids (PNAs) and proteins (Elmquist et al. 2001). It can enter mammalian and microbial cells and preferentially permeates and kills microbes; for example, it was described to kill *Mycobacterium smegmatis* at low micromolar doses at which normal human cells were not damaged (Nekhotiaeva et al. 2004; Palm et al. 2006).

Pep-1 is a chimeric peptide composed of the nuclear localization sequence of simian virus 40 large T antigen and of reverse transcriptase of human immunodeficiency virus. It has a hydrophobic tryptophan-rich domain, a spacer domain, and a hydrophilic lysine-rich domain and is characterized by an amphipathic α-helical structure (Henriques and Castanho 2004; Henriques et al. 2005; Morris et al. 2001). Pep-1 has a broad antimicrobial spectrum against Gram-negative and Gram-positive bacterial strains but weak antibacterial activity (Zhu et al. 2006). A bacteria-selective variant could be designed by replacing several glutamic acids with lysines. The modified peptide showed high activity (MIC 1–2 μM) against strains of Gram-positive and Gram-negative bacteria as well as against clinical isolates of multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) and methicillin-resistant *S. aureus* (MIC 1–8 μM) (Zhu et al. 2006). Notably, the peptide exhibits no hemolytic activity. For the mode of action, it was suggested that it occurs via formation of small channels that can transport ions or protons, and not by membrane disruption (Zhu et al. 2006).

Transportan (TP) is a 27-amino-acid chimeric peptide composed of the neuropeptide galanin and mastoparan-X linked by a lysine. It exhibits rapid and nonendocytotic uptake (Pooga et al. 1998) and was used for delivery of peptides, PNA oligomers or even intact proteins (Pooga et al. 2001). TP 10 is a 21-amino-acid deletion analog of the chimeric CPP transportan that contains the mastoparan sequence but lacks the toxicity of its parent compound. It can enter mammalian and microbial cells and preferably permeate and kill microbes such as *S. aureus* at low micromolar doses but does not damage human cells (Nekhotiaeva et al. 2004).

The cationic and amphipathic model peptide (MAP) is another CPP with antimicrobial properties. The uptake of the peptide again seems to be a combination of energy-dependent and energy-independent mechanisms, whereas the amphipathicity of the peptide is crucial for uptake (Oehlke et al. 1998; Scheller et al. 1999). It exhibits an

Table 3 Minimum inhibitory concentration (MIC) of selected cell-penetrating peptides

Peptide	MIC (μM)			References
	Gram-negative	Gram-positive	Fungi	
Tat (48–60)	2 (*E. coli* KCTC1682)	4 (*B. subtilis* KCTC1918)	24 (*S. cerevisiae* KCTC7296)	Zhu and Shin (2009b)
		4 (*S. aureus* KCTC1621)		Jung et al. (2006)
Penetratin	2 (*E. coli* KCTC1682)	0.5 (*B. subtilis* KCTC3068)	>25 (*S. cerevisiae* BY4741)	Zhu and Shin (2009a)
	25 (*E. coli* K12)	1 (*S. aureus* KCTC1621)		Palm et al. (2006)
		1 (*B. megaterium*)		
pVEC	25 (*E. coli* K12)	>10 (*B. subtilis* 168)	>25 (*S. cerevisiae* BY4741)	Palm et al. (2006)
		>10 (*S. aureus* RN4220)		Nekhotiaeva et al. (2004)
		1 (*B. megaterium*)		
Pep-1	32 (*E. coli* KCTC1682)	8 (*B. subtilis* KCTC3068)	Not reported	Zhu et al. (2006)
		64 (*S. aureus* KCTC1621)		
TP10	>10 (*E. coli* K12)	>10 (*B. subtilis*)	8 (*S. cerevisiae* BY4741)	Nekhotiaeva et al. (2004)
		4 (*S. aureus*)		
MAP	25 (*E. coli* K12)	1 (*B. megaterium*)	>25 (*S. cerevisiae*)	Palm et al. (2006)

antimicrobial effect against Gram-positive bacteria such as *B. megaterium* and Gram-negative bacteria such as *E. coli* in a low micromolar range. However, no antifungal activity against yeast *S. cerevisiae* has been observed (Palm et al. 2006).

Limitations

The examples described so far may lead to one question: do all CPPs share antimicrobial properties and may all AMPs be used as CPPs?

The fact is that, at high enough concentrations, also CPPs perturb membranes and become membrane permeabilizers (Palm et al. 2006), whereas it has been demonstrated that some AMPs are able to reach the cytoplasm without killing the whole microorganism. However, application in the other field would not be of real interest for all CPPs or AMPs. For instance, for some CPPs described, the antimicrobial effect is only achieved by applying high concentrations of the peptides, making their future use inefficient.

As mentioned before, CPPs are mostly defined to act in a transporter- and receptor-independent manner, whereas some AMPs such as pyrrhocoricin internalize via a receptor-mediated mechanism, or via a transporter-mediated mechanism in the case of apidaecin. Thus, like CPPs, they can be used as delivery vectors and translocate through cell membranes, but the uptake mechanisms are different from the conventional CPPs.

On the other hand, AMPs, to some extent, are unspecific and thus not only disturb bacterial, but rather destroy other cell membranes, too. This entails toxicity against the whole organism and makes their application as drug delivery vectors in vivo unfeasible. An example is the 26-amino-acid highly antimicrobial peptide melittin from the venom of honey bees. It is active in a micromolar range against Gram-negative and Gram-positive bacteria and is cytotoxic against mammalian cells (Blondelle and Houghten 1991). It acts via a channel-forming mechanism and is reported to be membranolytic (Hristova et al. 2001). Owing to its hemolytic properties, its use as a therapeutic drug-delivery agent is not possible (Blondelle and Houghten 1991).

Generally, it can be said that each peptide enters the cell following a different mechanism. Although the properties of AMPs and CPPs overlap to some extent, it has to be elucidated for each peptide if it is worth using as an AMP or CPP. Modifications in the sequence of the peptide can magnify the cell-penetrating or antimicrobial effect and have to be fitted for each peptide and aim.

Conclusions

This review provides evidence that some CPPs may also act as AMPs and that some AMPs may be applied as useful CPPs, an assumption also recently discussed by Henriques et al. (2006). Thus, we propose that these peptides cannot be stringently allocated to one or the other group. Future research will show whether the application of some CPPs as antimicrobials is realistic and applicable. Also, some of the known CPP motifs could be used to design new and better AMPs. In addition, the focus should be on some promising AMP-derived structures that are worth investigating and optimizing in more detail for their drug delivery characteristics.

Acknowledgments This work was supported by the Deutsche Forschungsgemeinschaft (DFG) within the project FOR 630 "Biological function of organometallic compounds."

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