Chapter 11
The Two-Peptide (Class-IIb) Bacteriocins: Genetics, Biosynthesis, Structure, and Mode of Action

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Abstract The two-peptide (class-IIb) bacteriocins consist of two different peptides, both of which are required to obtain high antimicrobial activity. These bacteriocins kill target-cells by inducing membrane-leakage and they seem to display some specificity with respect to the molecules they transfer across membranes. The genes encoding the two peptides of two-peptide bacteriocins are next to each other on the same operon. In the same or a nearby operon are genes encoding (i) the immunity protein that protects the bacteriocin-producer from its own bacteriocin, (ii) a dedicated ABC-transporter that exports the bacteriocin from cells and cleaves off the N-terminal bacteriocin leader sequence, and (iii) an accessory protein whose exact function has not been fully clarified. Some two-peptide bacteriocins appear to be produced constitutively, whereas the production of other two-peptide bacteriocins is regulated through a three-component regulatory system that consists of a peptide pheromone, a membrane-associated histidine protein kinase, and response regulators. It has recently been proposed that the two peptides of (some) two-peptide bacteriocins may form a membrane-penetrating helix-helix structure involving helix-helix interacting GxxxG-motifs present in all currently characterized two-peptide bacteriocins. It has also been suggested that the helix-helix structure interacts with an integrated membrane (transport) protein, thus inducing a conformational change in the protein, which in turn causes membrane-leakage. This proposed mode-of-action is similar to that of the pediocin-like (class-IIa) bacteriocins and lactococcin A, which bind to a part of the mannose phosphotransferase permease that is embedded in the cell membrane, thereby altering the conformation of the permease in a manner that causes membrane-leakage and cell death.

This chapter focuses on the two-peptide (class-IIb) bacteriocins. As the name suggests, the two-peptide bacteriocins are novel in that they consist of two very different peptides and optimal activity requires the presence of both peptides in about equal amounts (Garneau, Martin and Vederas 2002; Oppegård et al. 2007b; Nissen-Meyer et al. 2009, 2010). Since the first isolation of a two-peptide bacteriocin, lactococcin G, in 1992 (Nissen-Meyer et al. 1992), at least 15 two-peptide bacteriocins produced by lactic acid

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Lactococcin G	LcnG-α LcnG-β	GTWDDIGQGIGRVAYAWVGKAMGNMSDVNQASRINRKKKH KKWGWLAWVDPAYEFIKGFGKGAIKEGNKDKWKNI
Lactococcin Q	LcnQα LcnQβ	SIWGDIGQGVGKAAYAWVGKAMGNMSDVNQASRINRKKKH KKWGWLAWVEPAGEFLKGFGKGAIKEGNKDKWKNI
Enterocin 1071	Entα Entβ	ESVFSKIGNAVGPAAYWILKGLGNMSDVNQADRINRKKH GPGKWLPWLQPAYDFVTGIAKGIGKEGNKNKWKNV
Plantaricin E/F	PlnE PlnF	FNRGGYNFGKSVRHVVDAIGSVAGIRGILKSIR VFHAYSARGVRNNYKSAVGPADWVISAVRGFIHG
Plantaricin J/K	PlnJ PlnK	GAWKNFWSSLRKGFYDGEAGRAIRR RRSRKNGIGYAIGYAFGAVERAVLGGSRDYNK
Plantaricin S	Plsα Plsβ	RNKLAYNMGHYAGKATIFGLAAWALLA KKKKQSWYAAAGDAIVSFGEGFLNAW
Plantaricin NC8	PLNC8α PLNC8β	DLTTKLWSSWGYYIGKKARWNLKHPYVQF SVPTSVYTLGIKILWSAYKHRKTIEKSFNKGFYH
Lactacin F	LafA LafX	RNNWQTNVGGAVGSAMIGATVGGTIGPACAVAGAHYLPILWTGVTAATGGFGKIRK NRWGDTVLSAASGAGTGIKACKSFGPWGMAICGVGGAAIGGYFGYTHN
Brochocin-C	BrcA BrcB	YSSKDCLKDIGKGIGAGTVAGAAGGGLAAGIGAIPGAFVGAHFGVIGGSAACIGGLIGN KINWGNVGGSCVGGAVIGGALGGLGGAGGGCITGAIGSIWDQW
Thermophilin 13	ThmA ThmB	YSGKDCLKDMGGYALAGAGSGALWGAPAGGVGALPGAFVGAHVGAIAGGFACMGGMIGNKFN QINWGSVVGHIGGAIIGGAFSGGAAAGVGCLVGSGKAIINGL
ABP-118	Abp118α Abp118β	KRGPNCVGNFIGGIFAGAAAGVPLGPAGIVGGANIGMVGGALTCL KNGYGGSGNRWVHCGAGIVGGALIGAIGGPWSAVAGGISGGFTSCR
Salivaricin P	Sln1 Sln2	KRGPNCVGNFIGGIFAGAAAGVPLGPAGIVGGANIGMVGGALTCL KNGYGGSGNRWVHCGAGIVGGALIGAIGGPWSAVAGGISGGFASCH
Mutacin IV	NlmA NlmB	KVSGGEAVAAIGIGIGATASAAIGGLAGATLVTPYCVGTWGLIRSH DKQAADTFLSAVGGAASGFTYCASNGVWHPYIILAGCAGVGAVGSVVFPH
Lactocin 705	705α 705β	GMSGYIQGIPDFLKGYLHGISAANKHKKGRLGY GFWGGLGYIAGRVGAAYGHAQASANNHHSPING

Fig. 11.1 Amino acid sequences of two-peptide (class-IIb) bacteriocins. The GxxxG-motifs are marked with *black* background. Plantaricin Sβ and plantaricin NC8β have the GxxxG-like motifs AxxxA-and SxxxS-motif, respectively, instead of the GxxxG-motif. The two-peptide bacteriocins lactococcin MN (van Belkum et al. 1991), lactococcin MMT24 (Ghrairi et al. 2005), and leucocin H (Blom et al. 1999) are not included in the figure. Lactococcin MN has putative GxxxG-motifs, but is not included since only the sequences of the pre-forms are known and the cleavage site is unknown. Lactococcin MMT24 has not been sequenced and leucocin H has not been completely sequenced, but the partial sequence reveals a putative GxxxG-motif. The recently characterized two-peptide bacteriocin enterocin C (Maldonado-Barragan et al. 2009) is also not included. Its sequence is identical to enterocin 1071, except that enterocin C has an alanine residue instead of a threonine residue at position 17 in its β peptide (Maldonado-Barragan et al. 2009). References for the sequences are as follows: lactococcin G (Nissen-Meyer et al. 1992), lactococcin Q (Zendo et al. 2006), enterocin 1071 (Balla et al. 2000; Franz et al. 2002; Balla and Dicks 2005), plantaricin E/F and J/K (Diep, Håvarstein and Nes 1996; Anderssen et al. 1998), plantaricin S (Jiménez-Diaz et al. 1995; Stephens et al. 1998), plantaricin NC8 (Maldonado, Ruiz-Barba and Jiménez-Diaz 2003), lactacin F (Fremaux, Ahn and Klaenhammer 1993; Allison, Fremaux and Klaenhammer 1994), brochocin C (McCormick et al. 1998), thermophilin 13 (Marciset et al. 1997), ABP-118 (Flynn et al. 2002), salivaricin P (differs from ABP-118 in only two residues) (Barrett et al. 2007), mutacin IV (Qi, Chen and Caufield 2001), and lactocin 705 (Cuozzo et al. 2000). The figure is a modified version of figure 1 in Oppegård et al. (2008) and in Nissen-Meyer et al. (2010)

bacteria have been identified and characterized (Fig. 11.1). It should be noted that also two-peptide lantibiotics (i.e. class I bacteriocins) have been identified (Cotter, Hill and Ross 2005a, b), but they are not discussed in this chapter.

The Two-Peptide Bacteriocins Render Target-Cell Membranes Permeable to Small Molecules

All two-peptide bacteriocins whose mode-of-action has been analyzed, this includes lactacin F (Abee, Klaenhammer and Letellier et al. 1994), lactococcin G (Moll et al. 1996, 1998), thermophilin 13 (Marciset et al. 1997), plantaricin E/F (Moll et al. 1999), plantaricin J/K (Moll et al. 1999), and lactocin 705 (Castellano, Raya and Vignolo et al. 2003; Cuozzo et al. 2003), induce membrane-leakage in sensitive bacteria. Interestingly, the bacteriocins seem to display specificity with respect to the molecules they transfer across membranes, and they appear to some extent to vary in their specificities. For instance, both plantaricin E/F and plantaricin J/K render target-cell membranes permeable for monovalent ions, including H^+, but not for divalent ions such as Mg^{2+} and phosphate (Moll et al. 1999). Moreover, it seems that plantaricin E/F conducts cations with greater efficiency than plantaricin J/K and vice versa for anions (Moll et al. 1999). Also lactococcin G renders target-cell membranes permeable for different monovalent cations, such as Na^+, K^+, Li^+, Cs^+, Rb^+, and choline, but not to H^+ (in contrast to plantaricin E/F and plantaricin J/K), nor to divalent cations such as Mg^{2+} and anions such as phosphate (Moll et al. 1996, 1998). This capacity to discriminate between molecules they transfer through membranes suggests that two-peptide bacteriocins do not cause membrane leakage through simply a detergent-like disruption of membranes, but form (or trigger the formation of) rather sophisticated pores that show specificity with respect to transport of molecules. Furthermore, the high potency of two-peptide bacteriocins indicates that bacteriocin-induced membrane leakage depends on a low number of peptides, in contrast to what is expected if leakage is due to a detergent-like disruption of membranes.

The Two Peptides of Two-Peptide Bacteriocins Interact and Function as One Entity

The individual peptides of two-peptide bacteriocins often have features in common with one-peptide bacteriocins; they are usually (1) 30–50 residues long, (2) cationic, (3) membrane-active, (4) amphipathic and/or hydrophobic, and (5) synthesized as prepeptides with a 15- to 30-mer N-terminal leader sequence that is cut off by a dedicated ABC transporter upon export of the peptides from cells (Garneau et al. 2002; Oppegård et al. 2007b; Nissen-Meyer et al. 2009, 2010). Furthermore, one or both peptides of a few two-peptide bacteriocins [for instance, plantaricin E/F, plantaricin J/K (Anderssen et al. 1998), and lactacin F (Allison et al. 1994)] may separately have some – although very low – antimicrobial activity. Nevertheless,

two-peptide bacteriocins should not be considered as simply being two one-peptide bacteriocins that function in a synergistic manner. In fact, the individual peptides of many two-peptide bacteriocins do not exhibit any significant activity. For example, the two lactococcin G peptides exhibit no activity when assayed separately at concentrations up to 50 μM but are in combination active at pico- to nanomolar concentrations (Nissen-Meyer et al. 1992; Moll et al. 1996). Moreover, a peptide from a two-peptide bacteriocin shows high antimicrobial activity only when combined with the complementary peptide from the same two-peptide bacteriocin, or in some instances when combined with a peptide from a similar two-peptide bacteriocin. For example, the two lactococcin G peptides exhibit no activity when combined with either the E- or F-peptide of plantaricin E/F or the J- or K-peptide of plantaricin J/K (Anderssen et al. 1998). High activity is, however, attained when one of the lactococcin G peptides is combined with the complementary peptide from enterocin 1071 or lactococcin Q (Zendo et al. 2006; Oppegård et al. 2007a, b), apparently because these three two-peptide bacteriocins have similar sequences: almost 60% sequence identity between lactococcin Q and enterocin 1071, and between lactococcin G and enterocin 1071, and almost 90% identity between lactococcin G and lactococcin Q, (Balla et al. 2000; Zendo et al. 2006; Oppegård et al. 2007a, b) (Fig. 11.1). The facts that (1) both complementary peptides are required to attain high antimicrobial activity, (2) peptides function together only if they belong to the same or a similar two-peptide bacteriocin, (3) the genes encoding the two peptides of two-peptide bacteriocins are next to each other on the same operon (for references, see legend to Fig. 11.1), and (4) there is only one immunity gene for each two-peptide bacteriocin (for references, see legend to Fig. 11.1) clearly demonstrate that the two peptides of two-peptide bacteriocins should in fact be thought of as one functional antimicrobial entity. Furthermore, circular dichroism (CD) structural studies done on three two-peptide bacteriocins (plantaricin E/F, plantaricin J/K, and lactococcin G) revealed that the two peptides of these bacteriocins interact and structure each other upon exposure to target membranes (Hauge et al. 1998b, 1999). This is probably also the case for the peptides that constitute lactococcin Q, enterocin 1071, and enterocin C, since these three bacteriocins have more than 55% sequence identity with lactococcin G (see Fig. 11.1). The synergistic action of the two peptides of many, if not all, two-peptide bacteriocins is thus due to the fact that the two peptides interact with each other and form one antibacterial entity, rather than that they act separately at two different sites on target cells. It is not clear at what exact stage the two peptides interact with each other. It does not, however, occur before the peptides come in contact with the target cells, since both peptides of two-peptide bacteriocins are unstructured (and thus do not interact and structure each other) in the absence of membrane-like entities (Hauge et al. 1998b, 1999; Fimland et al. 2008; Rogne et al. 2008, 2009). Moreover, antimicrobial activity of lactococcin G is attained when sensitive cells are first treated with one of the lactococcin G peptides, washed, and subsequently treated with the other lactococcin G peptide, indicating that these peptides need not interact with each other before they interact with target cells. No antimicrobial activity is, however, obtained when target cells that have been treated with one of the lactococcin G peptides are mixed with target cells that have been

treated with the other lactococcin G peptide (Moll et al. 1998), indicating that the peptides are not able to diffuse to another cell once bound to the cell surface.

Although two-peptide bacteriocins are obviously not two synergistically acting one-peptide bacteriocins, one might speculate that some two-peptide bacteriocins have evolved from two one-peptide bacteriocins (Oppegård et al. 2007b). If two one-peptide bacteriocins that function in a synergistic manner were produced by the same bacteria, there could be a selection pressure for improvement of the synergistic effect – possibly at the expense of the activity of the individual peptides. This might in turn create selection pressure for genetically linking the two peptides, with the formation of a two-peptide bacteriocin.

Genes and Proteins Required for Production of Two-Peptide Bacteriocins

At least five genes found in either one or two operons are needed for the production of two-peptide bacteriocins. These genes are (1) the two structural genes encoding the preforms of the two peptides that constitute the bacteriocin, (2) the immunity gene encoding the immunity protein that protects the bacteriocin producer from its own bacteriocin, (3) the gene encoding the dedicated ABC transporter that exports the bacteriocin from cells, and (4) a gene encoding an accessory protein whose function has not been fully clarified, which might be involved in immunity and or export of the bacteriocin. For all two-peptide bacteriocins that have been characterized genetically, the two structural genes are found to be next to each other on the same operon and the two peptides that constitute these bacteriocins are thus thought to be produced in about equal amounts. This operon also contains the immunity gene, whereas the genes that encode the dedicated ABC transporter and the accessory protein are either also in this operon [this is the case for lactococcin G (Nes et al. 1995)] or on a separate – but nearby – operon [this is the case for plantaricin E/F, plantaricin J/K, and enterocin 1071 (Diep et al. 1996, 2009; Balla et al. 2000; Franz et al. 2002; Balla and Dicks 2005)].

Most, if not all, two-peptide bacteriocins (Fig. 11.1) are initially synthesized with a 15- to 30-mer N-terminal leader sequence of the double-glycine type. This leader sequence is apparently cut off at the C-terminal side of two glycine residues by the dedicated ABC transporter when the bacteriocin is exported out of cells. A special feature of the dedicated ABC transporter is an N-terminal region of approximately 150 residues that is absent in other ABC transporters. Studies using the N-terminal region of the ABC transporter that exports lactococcin G revealed that this region specifically cuts off the lactococcin G leader sequence at the C-terminal side of the double-glycine motif (Håvarstein et al. 1995). The bacteriocin leader sequence consequently appears to facilitate interactions with the ABC transporter, as has been shown to be the case for some one-peptide bacteriocins (van Belkum et al. 1997; Horn et al. 1998). The leader sequence might possibly also function to keep the bacteriocin inactive until it has been exported.

It is not entirely clear how immunity proteins protect cells from two-peptide bacteriocins. Recent results have indicated, however, that the immunity proteins for lactococcin G and enterocin 1071 function through a cellular component, since the ability of these immunity proteins to recognize their cognate bacteriocin is highly dependent on the cells in which the immunity proteins are expressed (Oppegård et al. 2010). Also, the functionality of the immunity proteins for the one-peptide pediocin-like bacteriocins depends on a cellular component (Fimland et al. 2002a; Johnsen et al. 2004), and this component appears to be the bacteriocin receptor, the mannose phosphotransferase permease (Dalet et al. 2001; Gravesen et al. 2002; Ramnath et al. 2004; Diep et al. 2007). It has thus been hypothesized that the cellular component through which the lactococcin G and enterocin 1071 immunity proteins function might be the receptors for these two bacteriocins. This receptor has, however, not yet been identified. The immunity proteins for two-peptide bacteriocins might possibly interact with the cellular component via the cell membrane, since structure predictions indicate that some immunity proteins for two-peptide bacteriocins have transmembrane helices. The putative immunity proteins of the two-peptide bacteriocins brochocin-C (McCormick et al. 1998; Nes et al. 2002), plantaricin S (Stephens et al. 1998; Nes et al. 2002), and thermophilin 13 (Marciset et al. 1997; Nes et al. 2002) are predicted to contain two transmembrane helices, while the immunity proteins of lactococcin G (Nes et al. 1995, 2002), lactococcin MN (van Belkum et al. 1991; Nes et al. 2002), plantaricin E/F (Diep et al. 1996; Nes et al. 2002) and plantaricin J/K (Diep et al. 1996; Nes et al. 2002) may contain 4–5 transmembrane helices. The number of transmembrane helices thus seems to differ, but a common mechanism for bacteriocin-immunity-involving interactions with membrane proteins – as is the case for the immunity proteins for the pediocin-like (class-IIa) bacteriocins (Diep et al. 2007) – may nevertheless exist.

Production of Two-Peptide Bacteriocins in Some Bacteria Is Regulated Through Three-Component Regulatory Systems

Some two-peptide bacteriocins appear to be produced constitutively, while the production of other two-peptide bacteriocins [such as NC8 (Maldonado et al. 2004), ABP-118 (Flynn et al. 2002), plantaricin E/F, plantaricin J/K (Diep et al. 1995, 1996, 2003)] is regulated transcriptionally through a three-component regulatory system that consists of a peptide pheromone, a membrane-associated histidine protein kinase, and response regulators (Kleerebezem and Quadri 2001). Upon export from bacteria, the peptide pheromone interacts with the membrane-associated histidine kinase and thus triggers the kinase to phosphorylate the intracellular response regulator, thereby enabling the response regulator to activate the operons that are required for bacteriocin synthesis and secretion (Kleerebezem and Quadri 2001).

The regulation of bacteriocin production by a three-component regulatory system has been thoroughly studied in *Lactobacillus plantarum* C11 (Diep et al.

1995, 1996, 2003). This strain produces the two two-peptide bacteriocins plantaricin E/F and plantaricin J/K, as well as the peptide pheromone plantaricin A that triggers the production of the two bacteriocins (Diep et al. 1995, 1996, 2003; Anderssen et al. 1998; Hauge et al. 1998a). The gene encoding plantaricin A is positioned in a regulatory operon that also contains the genes that encode the histidine protein kinase and two response regulators. The production of plantaricin E/F and plantaricin J/K is apparently triggered when the concentration of plantaricin A attains a threshold value due to high cell density. In addition to inducing bacteriocin production, plantaricin A stimulates its own production. An autoinduction loop is thus formed, resulting in rapid enhancement of the transcription of all genes involved in the production of plantaricin E/F and plantaricin J/K.

Signal transduction is evidently initiated by the binding of plantaricin A to the membrane-associated histidine protein kinase, and this induces the kinase to phosphorylate the two response regulators. The phosphorylated response regulators then activate the genes that encode plantaricin E/F and plantaricin J/K, as well as the gene that encodes plantaricin A (Diep et al. 2003). Structure and mode-of-action studies have revealed a novel membrane-interacting mechanism by which plantaricin A (and possibly other membrane-active peptide pheromones) functions (Kristiansen et al. 2005). Plantaricin A apparently initially interacts in a nonchiral manner with membrane lipids. This interaction induces α-helical structuring in a region of the peptide (Kristiansen et al. 2005), thus enabling the peptide to interact in a chiral manner with the histidine protein kinase receptor in or near the membrane–water interface.

The Structure of Two-Peptide Bacteriocins

The structures of three two-peptide bacteriocins (lactococcin G, plantaricin E/F, and plantaricin J/K) have been studied by use of circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy (Hauge et al. 1998b, 1999; Fimland et al. 2008; Rogne et al. 2008, 2009). The CD studies revealed that the peptides of these three bacteriocins are unstructured in aqueous solution (Hauge et al. 1998b, 1999). Moreover, there are no apparent structural interactions between the peptides. Helical structuring is, however, attained upon exposing the peptides individually to membrane-like environments, such as solutions containing micelles or liposomes (Hauge et al. 1998b, 1999; Fimland et al. 2008; Rogne et al. 2008, 2009). Furthermore, additional structuring is attained upon jointly exposing complementary peptides to membrane-like entities such as liposomes, suggesting that the peptides of two-peptide bacteriocins interact in a structure-inducing manner upon contact with target-cell membranes (Hauge et al. 1998b, 1999).

Interestingly, all presently characterized two-peptide bacteriocins contain GxxxG motifs (Fig. 11.1). This motif is often involved in helix–helix interactions between transmembrane helices in membrane proteins (Senes et al. 2000, 2001, 2004). When a GxxxG motif is present in an α-helix, the two glycine residues in the motif will be on the same side of the helix and thus form a flat surface that

permits close interhelical contact. This enables optimal interhelical van der Waals interactions and formation of stabilizing interhelical backbone Cα–H···O hydrogen bonds (Senes et al. 2001). The high helical content and presence of GxxxG motifs in the peptides of two-peptide bacteriocins thus suggest that a membrane-penetrating helix–helix structure involving GxxxG motifs may be a dominant structural element in many, if not all, two-peptide bacteriocins.[1]

Structure and Membrane-Orientation of Lactococcin G

Lactococcin G is produced by several strains of *Lactococcus lactis* and is presently the two-peptide bacteriocin that is best characterized (Nissen-Meyer et al. 1992; Moll et al. 1996, 1998; Hauge et al. 1998b; Oppegård et al. 2007a, 2008; Rogne et al. 2008). The bacteriocin consists of the 39-mer α-peptide (often termed LcnG-α) and the 35-mer β-peptide (often termed LcnG-β) (Fig. 11.1). CD- and NMR-structural studies revealed extensive α-helical structuring in both the α- and β-peptides upon exposure to either dodecylphosphocholine (DPC) micelles or the structure-inducing compound trifluoroethanol (TFE) (Hauge et al. 1998b; Rogne et al. 2008). Based on the NMR structures of the two peptides (Rogne et al. 2008) as well as on recent mutagenesis studies (Oppegård et al. 2008), a structural model of lactococcin G has been proposed (Oppegård et al. 2008; Rogne et al. 2008; Nissen-Meyer et al. 2009, 2010). The structural model is expected to be valid also for lactococcin Q, enterocin C, and enterocin 1071, because of their sequence similarities to lactococcin G. The structural model entails that the α- and β-peptides form a membrane-spanning helix–helix structure that is stabilized by helix–helix interacting GxxxG motifs and that the two peptides lie in a parallel and staggered fashion relative to each other (Oppegård et al. 2008; Rogne et al. 2008; Nissen-Meyer et al. 2009, 2010) (Fig. 11.2). The helix–helix region includes the N-terminal part of the α-peptide (covering approximately residues 3–22) and the C-terminal part of the β-peptide (covering approximately residues 13–32) (Fig. 11.2). Furthermore, it is proposed that the positively charged and unstructured C-terminal end (Arg-Lys-Lys-Lys-His; residues 35–39) of the α-peptide is pulled across the target-cell membrane by the transmembrane potential, while the relatively unstructured tryptophan-rich N-terminal part (covering approximately residues 1–10) of the β-peptide remains in the outer membrane interface (Fig. 11.2). The α- and β-peptides thereby form a helix–helix structure that spans the target-cell membrane with the N-termini of the two peptides outside and the C-termini inside the cell (Fig. 11.2). This structural model can account for recent results that imply that the lactococcin G immunity protein recognizes both a

[1] Although the two-peptide bacteriocin brochocin-C contains several GxxxG motifs (Fig. 11.1), it has been reported that brochocin-C – and possibly also thermophilin 13, since its sequence is similar to that of brochocin-C (Fig. 11.1) – might contain β-sheet structure (Garneau et al. 2003).

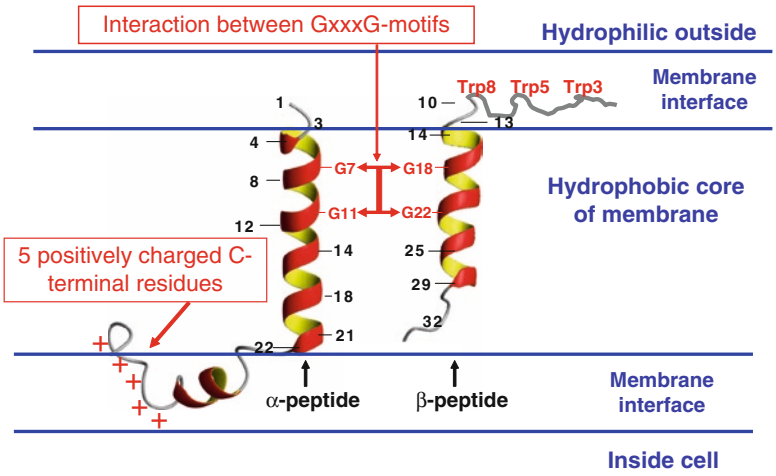

Fig. 11.2 Cartoon presentation of the proposed structural model of lactococcin G and its orientation in cell membranes. The two peptides interact through the G_7xxxG_{11} motif in the α-peptide and the $G_{18}xxxG_{22}$ motif in the β-peptide and form a transmembrane helix–helix structure. The tryptophan residues in the structurally flexible N-terminal region of the β-peptide are in or near the outer membrane interface, whereas the positively charged and structurally flexible C-terminal end of the α-peptide is forced through the membrane by the transmembrane potential (negative inside). The figure is from Nissen-Meyer et al. (2010)

sequence in the N-terminal part (residues 1–13) of the α-peptide and a sequence on the C-terminal side of residue 13 in the β-peptide (Oppegård et al. 2010). In the proposed structure, these regions in the α- and β-peptides are adjacent to each other, and this would thus enable the two regions to simultaneously interact with the immunity protein.

Structure of Plantaricin J/K

Also, the three-dimensional structures of the 25-mer J-peptide and 32-mer K-peptide of plantaricin J/K have been studied by CD and NMR spectroscopy (Rogne et al. 2009). Upon exposure to either DPC micelles or structure-inducing TFE, the J-peptide has an N-terminal amphipathic helical region from residue 3 to 15, whereas the K-peptide has a central amphipathic helical region from residue 9 to 24. There is one GxxxG motif ($G_{13}xxxG_{17}$) in the J-peptide and two (G_9xxxG_{13} and $G_{13}xxxG_{17}$) in the K-peptide (Fig. 11.1). Replacements of glycine residues in these peptides with large residues were very detrimental when the glycine residues were part of the $G_{13}xxxG_{17}$ motifs in the J and K peptides, whereas the replacements were fairly well tolerated when they were outside these two $G_{13}xxxG_{17}$ motifs (Rogne et al. 2009). The mutagenesis data and the NMR structures are consistent with a structural model in which also the J and K peptides of plantaricin J/K interact and

form a transmembrane helix–helix structure involving their $G_{13}xxxG_{17}$ motifs (Rogne et al. 2009). The results do not, however, reveal which relative orientation of the two peptides, the parallel or the antiparallel, is the most probable orientation (Rogne et al. 2009).

Structure of Plantaricin E/F

CD and NMR analysis of the 33-mer E-peptide and 34-mer F-peptide of plantaracin E/F (in the presence of micelles) revealed that the E-peptide has two helical regions separated by a flexible GxxxG motif ($G_{20}xxxG_{24}$), while the F-peptide has one long helical region (Fimland et al. 2008). The E-peptide has altogether two GxxxG motifs (G_5xxxG_9 and $G_{20}xxxG_{24}$) that might be involved interpeptide helix–helix interactions, whereas the F-peptide has one such motif ($G_{30}xxxG_{34}$). As is the case for lactococcin G, it has been proposed that the E- and F-peptides of plantaricin E/F interact in a staggered and parallel fashion relative to each other and form a transmembrane helix–helix structure that involves their GxxxG motifs (Fimland et al. 2008). There are, however, alternatives to how these GxxxG motifs might combine. It remains to be determined which of the possible alternatives are most likely.

Bacteriocins as Peptide Ligands that Interact with Membrane-Associated Receptors

Structure–function studies on the one-peptide pediocin-like (class-IIa) bacteriocins have shown that these bacteriocins have in their C-terminal half a helix-containing segment that penetrates into target-cell membranes (Fregeau Gallagher et al. 1997; Wang et al. 1999; Uteng et al. 2003; Haugen et al. 2005; Fimland et al. 2005; Drider et al. 2006; Nissen-Meyer et al. 2009). The membrane-penetrating helix-containing region is involved in determining the target-cell specificity of these bacteriocins and is also the region that is recognized by their immunity proteins (Fimland et al. 1996, 2002b, 2005; Johnsen et al. 2005; Drider et al. 2006; Nissen-Meyer et al. 2009). It has also been shown that the pediocin-like bacteriocins kill target cells by interacting with the membrane-associated mannose phosphotransferase permease (Diep et al. 2007). A part, probably the membrane-penetrating helix, of these bacteriocins apparently binds to the permease subunits (the MptC and/or MptD subunits) that are embedded in the membrane, thereby altering the conformation of the permease in a manner that leads to membrane leakage and cell death. Immunity proteins that protect cells from pediocin-like bacteriocins sense the altered conformation and bind to the bacteriocin–permease complex, thereby preventing membrane leakage (Diep et al. 2007). The fact that lactococcin A, a one-peptide class IId bacteriocin that is entirely different from the pediocin-like bacteriocins, functions in a similar manner (i.e., by binding to the membrane-embedded

mannose permease subunits) (Diep et al. 2007), suggests that several very different membrane-active peptide bacteriocins may induce membrane leakage in basically the same way; the common theme is that membrane leakage is caused by structural alterations in an integrated target-cell membrane (transport) protein and that these structural alterations are triggered by interactions between the target-cell membrane protein and a membrane-penetrating helical segment of the bacteriocin. For two-peptide bacteriocins, these interactions might involve the binding of the transmembrane helix–helix structure to an integrated membrane protein/receptor. A membrane-associated receptor for a two-peptide bacteriocin has, however, yet to be identified. It has nevertheless been proposed that the lactococcin G immunity protein interacts with lactococcin G via a lactococcin G receptor in an analogous manner as the immunity proteins for the pediocin-like bacteriocins, since the functionality of the lactococcin G immunity protein depends on a cellular component (Oppegård et al. 2010) and this component might well be the lactococcin G receptor. It should be noted that some studies suggest that some two-peptide bacteriocins, in particular thermophilin 13 and brochocin-C, might not need membrane receptors to exert their activity (Marciset et al. 1997; Gao et al. 1999). It is important that more extensive structure–function studies on peptide bacteriocins are carried out, since that will give us even better insight into how peptide bacteriocins function and reveal structural features that are especially important for their potency. Such insight is invaluable for rational design and construction of novel antibacterial peptides that may especially be useful for medical and biotechnological applications.

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