The C-terminal Domain of Pediocin-like Antimicrobial Peptides (Class IIa Bacteriocins) Is Involved in Specific Recognition of the C-terminal Part of Cognate Immunity Proteins and in Determining the Antimicrobial Spectrum*

Received for publication, November 10, 2004, and in revised form, December 17, 2004 Published, JBC Papers in Press, December 17, 2004, DOI 10.1074/jbc.M412712200

Line Johnsen‡, Gunnar Finland, and Jon Nissen-Meyer

From the Program for Biochemistry and Molecular Biology, Department of Molecular Biosciences, University of Oslo, Oslo 0316, Norway

The pediocin-like bacteriocins contain two domains: a cationic N-terminal β-sheet domain that mediates binding of the bacteriocin to the target cell surface and a more hydrophobic C-terminal hairpin-like domain that penetrates into the hydrophobic part of the target cell membrane. The two domains are joined by a hinge, which enables movement of the domains relative to each other. In this study, 12 different hybrid bacteriocins were constructed by exchanging domains between 5 different bacteriocins. The hybrid bacteriocins were by and large highly potent (i.e. similar potencies as the parental bacteriocins) when constructed such that the recombination point was in the hinge region, indicating that the two domains function independently. The use of optimal recombination points was, however, crucial. Shifting the recombination point just one residue from the hinge could reduce the activity of the hybrid by 3–4 orders of magnitude. Most interestingly, the active hybrids displayed target cell specificities similar to those of the parental bacteriocin from which their membrane-penetrating C-terminal hairpin domain was derived. The results also indicate that the negatively charged aspartate residue in the hinge of most pediocin-like bacteriocins interacts with the C-terminal hairpin domain, perhaps by interacting with the positively charged residue that is present at one of the last three positions in the C-terminal end of most pediocin-like bacteriocins. Bacteria that produce pediocin-like bacteriocins also produce a cognate immunity protein that protects the producer from being killed by its own bacteriocin. Four different active hybrid immunity proteins constructed by exchanging regions between three different immunity proteins were tested for their ability to confer immunity to the hybrid bacteriocins. The results showed that the C-terminal half of the immunity proteins contains a region that directly or indirectly specifically recognizes the membrane-penetrating C-terminal hairpin domain of pediocin-like bacteriocins. The implications these results have on how pediocin-like bacteriocins and their immunity proteins interact with cellular specificity determinants (for instance a putative bacteriocin receptor) are discussed.

Many Gram-positive bacteria produce ribosomally synthesized antimicrobial peptides, often termed bacteriocins. One important and well studied class of bacteriocins is the pediocin-like bacteriocins produced by lactic acid bacteria (1, 2). At least 20 different pediocin-like bacteriocins have been characterized (1–3). These bacteriocins are all cationic, display anti-Listeria activity, and kill target cells by permeabilizing the cell membrane (4, 5). They contain between 37 and 48 residues and have very similar primary structures. Despite their extensive sequence similarity, they differ markedly in their target cell specificity (6, 7). This difference in target cell specificity combined with their extensive sequence similarity makes the group of pediocin-like bacteriocins a well suited model system for analyzing the relationship between the structure and the target cell specificity of membrane-permeabilizing cationic antimicrobial peptides.

Based on their primary structures, the peptide chains of pediocin-like bacteriocins may be divided roughly into two regions: a hydrophilic, cationic and highly conserved N-terminal region, and a less conserved hydrophobic/amphiphilic C-terminal region (8). NMR studies indicate that the N-terminal region forms a three-stranded antiparallel β-sheet supported by a conserved disulfide bridge (9, 10). This N-terminal β-sheet region is followed by a central amphiphilic α-helix and this in turn by a rather extended C-terminal tail that folds back onto the central α-helix, thereby creating a hairpin-like structure in the C-terminal half (9–11). There is a flexible hinge between the β-sheet N-terminal region and the hairpin-like C-terminal region, and one thus obtains two domains that may move relative to each other (9). The cationic N-terminal β-sheet domain appears to mediate binding of the pediocin-like bacteriocins to the target cell surface through electrostatic interactions (12, 13), whereas the more hydrophobic and amphiphilic C-terminal hairpin domain apparently penetrates into the hydrophobic part of the target cell membrane, thereby mediating leakage through the membrane (3, 8, 14). The hinge apparently provides the structural flexibility that enables the C-terminal hairpin domain to dip into the hydrophobic part of the membrane (Ref. 9 and Fig. 1).

Genes encoding pediocin-like bacteriocins are generally co-transcribed with/or in close vicinity to a gene encoding a cognate immunity protein that protects the bacteriocin producers from their own bacteriocin (15–20). The primary structures of at least 17 immunity proteins of pediocin-like bacteriocins have been deduced from DNA sequences (21). These immunity proteins consist of 88–115 amino acid residues, and they display 5–85% sequence similarities (7). The proteins show a high degree of specificity with respect to the bacteriocin they recog-

This paper is available on line at http://www.jbc.org
nize, although there may in some cases be some cross-immunity (i.e. some immunity proteins render cells immune to a few pediocin-like bacteriocins in addition to their cognate bacteriocin) (21). The C-terminal half of the immunity proteins apparently contains a region that is involved in specific recognition of the bacteriocin to which they confer immunity (22). The mode of action of the immunity proteins is not known, but it has been suggested that they may act by disturbing the interaction between the bacteriocin and a (putative) membrane-located bacteriocin receptor (18, 23, 24). The immunity proteins for the two pediocin-like bacteriocins mesentericin Y105 and carnobacteriocin B2 have been shown to be located intracellularly, a small proportion (about 1%) possibly being associated with the cell membrane (16, 18). The recently reported NMR solution structure of the immunity protein for carnobacteriocin B2 has revealed that the protein consists of an antiparallel four-helix bundle (helix 1–4) with the C-terminal region (containing a fifth helix and an extended strand) being packed approximately in a perpendicular manner across helix 3 and 4 (25).

It has been proposed that the C-terminal region of pediocin-like bacteriocins is important in determining their target cell specificity because pediocin-like variants that are altered in the C-terminal region are derived indicates that the C-terminal hairpin domain contains important specificity determinants (27). Finally, the fact that hybrid bacteriocins containing N- and C-terminal regions from different pediocin-like bacteriocins have relative target cell specificities similar to that of the bacteriocins from which the C-terminal region is derived indicates that the C-terminal domain contains important specificity determinants (8). However, with one exception, all of the hybrid bacteriocins in this latter study (8) were 100–1,000 times less potent than the parental bacteriocins. Thus only relative specificities were determined, and this introduced some uncertainty into the interpretation of the results using hybrid bacteriocins (8). The three-dimensional structure of pediocin-like bacteriocins suggests that more optimal hybrid bacteriocins may be constructed if one uses the hinge as the recombination point and thereby exchange complete domains between bacteriocins. In this study, we have constructed potent hybrid bacteriocins using this approach and used these to show that the C-terminal membrane-penetrating hairpin domain is in fact the major specificity determinant. Moreover, using hybrid immunity proteins, we show that the C-terminal half of cognate immunity proteins specifically recognizes the C-terminal hairpin domain.

**Experimental Procedures**

**Bacterial Strains, Plasmids, and Media**—The hybrid bacteriocins Sak/Ped-17/15, En/tak/Sak-17/18, Sak/Ent-17/18, Sak/Leu-17/18, Leu/Sak-16/17, Leu/Sak-17/18, Cur/Sak-17/18, Cur/Sak-18/19, Sak/Cur-16/17, Sak/Cur-17/18, and Sak/Cur-18/19 (for a description of these hybrid bacteriocins, see “Results”) were produced by Lactobacillus sake LB790 transformed with a plasmid containing the hybrid bacteriocin gene. The wild-type bacteriocin producer strains used in this study were Lactobacillus curvatus LTH1174 producing curvacin A (28), Enterococcus faecium CTC492 producing enterocin A (29), Leuconostoc mesenteroides 6 producing leuconcin A (30) and Pediococcus acidilactici NCDO 2722 producing pediocin PA-1 (31). Sakacin P was produced by the two-plasmid L. sake LB790(pSAK20/pSSFP2) heterologous expression system described earlier (32).

The indicator strains used for (hybrid) bacteriocin activity measurements were L. sake NCDO 2714, Carnobacterium mesenteroides NCDO 3352, Pediococcus pentosaceus LGM 2722, and Lactobacillus sp. strain LGM 2003. L. sake NCDO 2714 and Enterococcus faecalis NCDO 581 containing a plasmid with or without an inserted (hybrid) immunity gene were used as indicator strains for assaying (hybrid) bacteriocin activity against (hybrid) immunity proteins. All LAB strains were grown in MRS broth (Oxoid) at 30°C. Agar plates for all LAB transformants were made by adding 1.5% agar to MRS broth. Selective antibiotic concentrations were used for the plasmids used as templates in the PCRs, pEAIM, pLIM, and pORFY, are

**PCR and DNA Sequencing**—Eurogentec (Belgium) produced all oligonucleotides used in this study. The hybrid immunity genes and the hybrid bacteriocin genes were amplified by PCR using a standard amplification protocol on a PTC-200 Peltier Thermal Cycler (MJ Research). The DNA sequences of the cloned hybrid bacteriocin genes and the hybrid immunity genes were verified by automated DNA sequence determination, using a MegaBase DNA analysis system and the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences).

**Construction of Hybrid Bacteriocin Expression Plasmids and Plasmids Containing Hybrid Immunity Genes**—To express the hybrid bacteriocins, a BglII restriction site was introduced by PCR between the genes pedD and pedC in the pMC117 (37) plasmid, which resulted in the plasmid pLJ100. PCR fragments containing the pediocin leader sequence, the hybrid bacteriocin sequence, and the immunity sequence belonging to the latter part of the hybrid bacteriocin were cloned in pLJ100 using the SacI and Bam/HI/BglII sites of fragments and vector. The resulting plasmids contained an operon consisting of the genes hybXY (pediocin leader and hybrid bacteriocin XY), imY (immunity sequence belonging to the former part of the hybrid bacteriocin), and pedC (which specifies a 174-amino acid membrane protein required for pediocin secretion), and pedD (codes for a protein that belongs to the ATP-dependent translocators family and which cleaves off the pediocin leader) (20, 38). PCR fragments containing the hybrid immunity genes, entDorf-im, entDorf-im, entDorf-im, leuorf-im, leuorf-im, and orfD-and-im, with flanking SacI and XbaI sites, were constructed using the PCR megaprimer method and cloned into the SacI and XbaI sites of pMG36e. The plasmids used as templates in the PCRs, pEAIm, pLIM, and pORFY, are described by Dayem et al. (16).

**Production, Synthesis, and Purification of (Hybrid) Bacteriocins**—Enterocin A, leuconcin A, pediocin PA-1, curvacin A, and sakacin P and 11 hybrid bacteriocins were purified to homogeneity from 0.5- or 1.0-liter cultures of their producer strains by cation exchange and reverse
phase chromatography, as described previously (39). The purity of the bacteriocins was analyzed by analytical chromatography on a HPLC column (Amersham Biosciences) using the SMART chromatography system (Amersham Biosciences) and water/2-propanol containing 0.1% (v/v) trifluoroacetic acid, as the mobile phase. Ped/Sak-21/22 was synthesized using an Applied Biosystems model 430A peptide synthesizer and the standard tert-butoxycarbonyl synthesis protocol of the manufacturer and purified as described previously (8). The primary structure of all the hybrid bacteriocins was confirmed by mass spectrometry on a Voyager-DE RP matrix assisted laser desorption time-of-flight mass spectrometer (Perseptive Biosystems); 3,5-dimethoxy-4-hydroxycinnamic acid was used as matrix. Correct disulfide bridge formation for peptides that contained four cysteine residues was confirmed by cleavage of the peptides with endoproteinase Asp-N (Roche Applied Science) followed by analysis of the resulting fragments with mass spectrometry.

Bacteriocin Assay—Bacteriocin activity was measured using a microtiter plate assay system, essentially as described previously (40). Each well of a microtiter plate contained 200 μl of culture medium with bacteriocin fraction at 2-fold dilutions and an indicator strain at an A610 of about 0.01 (inoculated from a 16–20-h overnight culture at 30 °C). The microtiter plate cultures were incubated overnight (14–16 h) at 30 °C, after which growth of the indicator strain was measured spectrophotometrically at 610 nm with a microtiter plate reader. The MIC was defined as the concentration of bacteriocin that inhibited growth of the indicator strain by 50%. The MIC values that are presented are the result of at least five independent measurements and had standard deviations of less than 50% of the value. Transformed indicator strains were grown in the presence of 5 g of erythromycin/ml to ensure that the plasmid containing the immunity gene was maintained in the cells. The protection pattern of each of the (hybrid) immunity proteins was judged by comparing MIC values for a strain expressing a hybrid immunity gene with MIC values for the same strain containing the unmodified pMG36e plasmid.

RESULTS AND DISCUSSION

Construction of Hybrid Bacteriocins by Combining the N- and C-terminal Domains of Various Pediocin-like Bacteriocins—Twelve different hybrid bacteriocins (Sak/Ped-17/18, Ped/Sak-21/22, Ent/Sak-17/18, Sak/Ent-17/18, Sak/Leu-17/18, Leu/Sak-16/17, Leu/Sak-17/18, Cur/Sak-17/18, Cur/Sak-18/19, Sak/Cur-16/17, Sak/Cur-17/18, and Sak/Cur-18/19) derived from five different wild-type bacteriocins (enterocin A, sakacin P, pediocin PA-1, leucocin A, and curvacin A) were constructed (Fig. 2). The numbers associated with each hybrid bacteriocin indicate the two residues that adjoin the recombination point (Fig. 2). With a few exceptions, the recombination point was chosen to be between residues 16 and 17 and/or between 17 and 18 when constructing the hybrid bacteriocins (Fig. 2) because structural studies (9) indicate that the hinge is at residue 17. Ped/Sak-21/22 was one exception because its recombination point was between residues 21 and 22 (Fig. 2). In a previous study this hybrid was shown to have the same potency as the parental bacteriocins (pediocin PA-1 and sakacin P) (8), and it was consequently retained for this study. It was obtained through peptide synthesis, in contrast to the other hybrid bacteriocins that were produced by L. sake LB790 transformed with a plasmid containing the hybrid bacteriocin gene.

Pediocin PA-1 and enterocin A contain two cysteine residues in their C-terminal domain in addition to the two cysteine residues present in the N-terminal domain of all pediocin-like bacteriocins (Fig. 2). Consequently, two of the hybrid bacteriocins, Sak/Ent-17/18 and Sak/Ped-17/18, contained four cysteine residues (Fig. 2). To ensure correct formation of disulfide bridges in these two hybrid bacteriocins, all hybrid bacteriocins (except Ped/Sak-21/22, which was synthesized) were con-
structed with a pediocin-leader and using a production plasmid that contained the genes \textit{pedC} and \textit{pedD} encoding the pediocin PA-1 secretion machinery (20, 38). In contrast to the secretion machinery for pediocin-like bacteriocins that contain only two cysteine residues, the pediocin PA-1 secretion machinery apparently makes correct disulfide bridges in pediocin-like bacteriocins that contain four cysteine residues (26). The plasmid constructs thus resulted in hybrid bacteriocins with correct disulfide bridges, as determined by endoproteinase Asp-N cleavage of Sak/Ent-17/18 and Sak/Ped-17/18 at the N-terminal side of the aspartate residue in position 17. The molecular masses (determined by mass spectrometry) of the fragments obtained were consistent with correct formation of disulfide bridges (between the two cysteine residues in the C-terminal half and between the two cysteine residues in the N-terminal half). The formation of incorrect disulfide bridges results in nearly inactive peptides (26), and the fact that the two peptides were active is also consistent with correct disulfide bridge formation.

To protect the transformed \textit{L. sake} LB790 strain from being killed by the hybrid bacteriocins, the hybrid bacteriocin genes were cotranscribed with the gene encoding the immunity protein for the parental bacteriocin from which the C-terminal domain of the hybrid was derived. These immunity genes were chosen because subgrouping of pediocin-like bacteriocins and their immunity proteins suggests that immunity proteins tend to provide immunity to bacteriocins that have similar C-terminal sequences (21).

The Hybrid Bacteriocins Are Active, and the C-terminal Domain Is Important in Determining Target Cell Specificity—To determine the potency of the hybrid bacteriocins and evaluate the relative importance of the N- and C-terminal domains (see Fig. 1) as target cell specificity determinants, all (hybrid) bacteriocins were tested against four different indicator strains. Most of the hybrid bacteriocins were highly potent (i.e. had potencies similar to that of the parental bacteriocins), and all of these displayed target cell specificity patterns similar to those of the parental bacteriocin from which their C-terminal domain was derived. Sak/Ped-17/18 had a specificity pattern similar to that of pediocin PA-1 but not to that of sakacin P, although the hybrid appeared to be somewhat more potent than pediocin PA-1 against three of the four indicator strains (Fig. 3a). The reciprocal hybrid, Ped/Sak-21/22, had in contrast a specificity pattern similar to that of sakacin P but not to that of pediocin PA-1, although it appeared overall to be slightly less potent than sakacin P (Fig. 3c). Similarly, Ent/Sak-17/18 had a specificity pattern and potency very similar to those of sakacin P (and Ped/Sak-21/22) but quite different from those of enterocin A (Fig. 3b). The reciprocal hybrid, Sak/Ent-17/18, on the other hand, resembled enterocin A more than sakacin P in that it showed the same high activity as enterocin A against strain a and was thus nearly 40 times more potent against this strain.

![Image](http://www.jbc.org/)

**Fig. 3.** a, graphic display of the activity of sakacin P, pediocin PA-1, and the hybrid bacteriocins Sak/Ped-17/18 and Ped/Sak-21/22 toward the indicator strains \textit{L. sake} NCDO 2714 (blue bars, inset a) \textit{C. piscicola} LMG 2332 (red bars, inset b), \textit{P. pentosaceus} LMG 2722 (yellow bars, inset c), and \textit{Lactobacillus} LMG 2003 (turquoise bars, inset d). The MIC is the concentration in \(\mu\)M which inhibited growth of the indicator strain by 50%. The results represent the averaged data from at least five experiments, and the S.D. values are indicated in each bar. b, graphic display of the activity of sakacin P, enterocin A-1, and the hybrid bacteriocins Ent/Sak-17/18 and Sak/Ent-17/18 toward the same indicator strains as indicated in the legend to 3a. The activity was quantified as described in the legend to 3a. c, graphic display of the activity of sakacin P, leucocin A, and the hybrid bacteriocins Cur/Sak-17/18 and Sak/Ent-17/18 toward the same indicator strains as indicated in the legend to 3a. The activity was quantified as described in the legend to 3a.
than sakacin P (Fig. 3b). It was also 1–2 orders of magnitude more potent against strains c and d than sakacin P, although it was not quite as potent (but within 1 order of magnitude) as enterocin A against these two strains (Fig. 3b). Interestingly, it was more potent against strain b (2–10 times more potent) than any of the other (hybrid) bacteriocins tested (Fig. 3, a–d).

Sak/Leu-17/18 had a specificity pattern and potency that were nearly identical to those of leucocin A and quite different from those of sakacin P, whereas the reciprocal hybrid Leu/Sak-17/18 had a specificity pattern somewhat similar to that of sakacin P, as the hybrid was clearly most (and about equally) active against the two most sakacin P-sensitive strains (see inset in Fig. 3c). Its potency, however, was about 5–20 times less than that of sakacin P, and the relative uncertainty in the measurements was large (see inset in Fig. 3c), thus making the interpretation of the results obtained with this hybrid uncertain. A slightly altered hybrid, Leu/Sak-16/17, was consequently constructed and analyzed. Both the potency and the specificity pattern of this latter hybrid were nearly identical to those of sakacin P but quite different from those of leucocin A (Fig. 3c). The Leu/Sak-16/17 and Leu/Sak-17/18 hybrids are identical except that the former has a negatively charged aspartate residue derived from sakacin P in the hinge at position 17, whereas the latter has a neutral (but polar) asparagine residue derived from leucocin A (Fig. 2). The negative charge of the aspartate residue is thus apparently crucial for the proper functioning of the Leu/Sak hybrids. This is perhaps not surprising because changing the aspartate in sakacin P to either asparagine or glutamine is known to reduce the potency of sakacin P 5–20 times (13). The negative charge of the aspartate residue and its orientation in space are consequently crucial for the proper functioning of sakacin P (13). Taken together, the results indicate that the negatively charged aspartate residue, which is conserved in all pediocin-like bacteriocins of subgroup 1 (Fig. 4), interacts with the C-terminal hairpin domain more so than with the N-terminal domain. The negative charge might possibly interact with the positively charged residue that is present at one of the last three positions in the C-terminal end of all subgroup 1 pediocin-like bacteriocins (see Fig. 4).

Interestingly, neither this C-terminal positive charge nor the negative charge at position 17 is well conserved in the pediocin-like bacteriocins of the two other subgroups (Fig. 4).

The Cur/Sak-17/18 hybrid also displayed a target cell specificity pattern and potency similar to those of the parental bacteriocin (sakacin P) from which its C-terminal domain was derived (Fig. 3d). Although the potency of the Cur/Sak-17/18 hybrid appeared to be slightly reduced compared with that of sakacin P (about half that of sakacin P; Fig. 3d), the hybrid clearly tolerated an asparagine residue (derived from curvacin A) in the hinge at position 17 to a much better extent than Leu/Sak-17/18 and sakacin P. Moving the recombination point one residue toward the C-terminal end, thereby constructing the Cur/Sak-18/19 hybrid (identical to Cur/Sak-17/18, except for an arginine residue at position 18 instead of a tryptophan residue; Fig. 2) reduced the activity by 3–4 orders of magnitude compared with Cur/Sak-17/18, illustrating the importance of using the correct recombination point. Construction of the reciprocal hybrids Sak/Cur-16/17 and Sak/Cur-17/18 resulted in removal of a central tryptophan residue that is present in all pediocin-like bacteriocins (Fig. 4). The central tryptophan residue positions itself in the water interface of target membranes and is thereby essential for the correct structuring and positioning of pediocin-like bacteriocins in membranes (3). As expected from this, the potency of the hybrids Sak/Cur-16/17 and Sak/Cur-17/18 was low (about 3 orders of magnitude lower than that of curvacin A; results not shown), and their specificities could consequently not be evaluated. A somewhat more active Sak/Cur hybrid with a central tryptophan residue was constructed by introducing the recombination point between residue 18 and 19. The Sak/Cur-18/19 hybrid thus obtained contained a sakacin P-derived central tryptophan residue at position 18 (Fig. 2) and was thus much more potent than Sak/Cur-16/17 and Sak/Cur-17/18 (about 200 times more potent). The hybrid was, however, still about 50-fold less potent.
TABLE I
(Hybrid) bacteriocin sensitivity of L. sake NCDO 2714 expressing various (hybrid) immunity genes
The results are presented as the -fold increase in MIC observed for strains expressing a (hybrid) immunity gene relative to MICs for strains containing only the control plasmid (pMG36e). The results represent the average data from at least five experiments, and the standard deviations were within 30% of the indicated values.

| (Hybrid) immunity protein | (Hybrid) bacteriocin |
|---------------------------|----------------------|
|                           | Sak/Ent-17/18 | Enterocin A | Ent/Sak-17/18 | Sakacin P | Leu/Sak-17/18 | Sak/Leu-17/18 | Leucocin A |
| EntA-im                   | 64          | 192         | 4            | 2         | 2            | 2            | 2          |
| LeuA-im                   | 1           | 1           | 1            | 1         | 32           | 16           |
| OrfY-im                   | 24          | 96          | 1            | 1         | 2            | 48           | 24         |
| EntA/LeuA-im              | 1           | 1           | 1            | 1         | 16           | 12           |
| LeuA/EntA-im              | 12          | 48          | 1            | 1         | 1            | 1            |
| OrfY/LeuA-im              | 1           | 1           | 1            | 1         | 32           | 8            |
| OrfY/EntA-im              | 1           | 1           | 1            | 1         | 1            | 1            |

TABLE II
(Hybrid) bacteriocin sensitivity of E. faecalis NCDO 581 expressing various (hybrid) immunity genes
The results are presented as the -fold increase in MIC observed for strains expressing a (hybrid) immunity gene relative to MICs for strains containing only the control plasmid (pMG36e). The results represent the average data from at least five experiments, and the standard deviations were within 40% of the indicated values.

| (Hybrid) immunity protein | (Hybrid) bacteriocin |
|---------------------------|----------------------|
|                           | Sak/Ent-17/18 | Enterocin A | Ent/Sak-17/18 | Sakacin P | Leu/Sak-17/18 | Sak/Leu-17/18 | Leucocin A |
| EntA-im                   | >500         | >500        | 64           | 24        | 12           | 6            | 4          |
| LeuA-im                   | 16           | 16          | 3            | 2         | 1            | 32           | 24         |
| OrfY-im                   | 64           | 64          | 4            | 2         | 2            | >500         | >500       |
| EntA/LeuA-im              | 2            | 3           | 1            | 1         | 16           | 12           |
| LeuA/EntA-im              | 48           | 48          | 1            | 1         | 1            | 1            |
| OrfY/LeuA-im              | 2            | 4           | 4            | 2         | 2            | >250         | >250       |
| OrfY/EntA-im              | >250         | >250        | 1            | 1         | 1            | 1            |

CONCLUDING REMARKS
Earlier studies have shown that the cationic N-terminal β-sheet domain of pediocin-like bacteriocins binds to the target cell surface and that the more hydrophobic C-terminal hairpin domain dips into the hydrophobic part of the cell membrane, the flexible hinge enabling movement of the two domains relative to each other (3, 8, 14; see Fig. 1). This model implies that the two domains function independently, which is consistent with the present results showing that potent hybrid bacteriocins may be constructed by joining N- and C-terminal domains from different pediocin-like bacteriocins. Use of optimal recombination points is, however, crucial for obtaining functional hybrids. With one exception (Ped/Sak-21/22), all of the hybrid bacteriocins constructed in an earlier study were 100–1,000 times less potent than the parental bacteriocins (8). The recombination points used in those constructs were not within the...
hinge region because the hybrids were designed without any information about the three-dimensional structure of pediocin-like bacteriocins (8). Even shifting the recombination point one residue from the hinge may reduce the activity by 3–4 orders of magnitude, as was the case when moving the recombination point from between residues 17 and 18 to between 18 and 19 when constructing Cur/Sak hybrids.

The affinity of a bacteriocin to the cell surface is expected to have an effect on the sensitivity of the cell to the bacteriocin. The N-terminal domain that interacts with the cell surface might consequently be expected to be a major specificity determinant. However, the surface of the target cells did not appear to discriminate between the different N-terminal domain variants because the present results show that the specificity-determining step involved the membrane-permeabilizing C-terminal hairpin domain rather than the surface binding N-terminal β-sheet domain. The specificity-determining step thus appears to involve interactions with lipids and/or proteins in the interphase and/or hydrophobic phase of the cell membrane. One protein candidate is the membrane-bound mannose phosphotransferase system permease, which must be expressed for cells to be sensitive to pediocin-like bacteriocins (42–46). It has been suggested that one of the subunits of the permease functions as a receptor or docking site for pediocin-like bacteriocins (42). For the permease to be involved in the specificity-determining step, the strain-dependent sequence variations in the permease must be large enough to cause sufficient strain-dependent variations in interactions between the permease and the various C-terminal hairpin domains. The fact that 15-mer fragments starting from the central hinge region and going toward the C-terminal end inhibit pediocin-like bacteriocins in a specific manner (6, 27, 47) suggests that this region of the C-terminal hairpin domain interacts with a bacteriocin receptor.

The C-terminal halves of the immunity proteins contain a region that directly or indirectly interacts specifically with the C-terminal hairpin domain of cognate bacteriocins. Although it has not been possible to demonstrate a direct bacteriocin-immunity protein interaction (10, 25), the existence of such an interaction cannot be excluded. It is possible that a direct interaction occurs only after the bacteriocin has become membrane-bound and thereby structured. Although the immunity proteins are not integrated membrane proteins involved in extensive membrane interactions (18, 22, 25), they might be loosely associated with the membrane, possibly as peripheral membrane proteins, thus enabling them to interact with their cognate bacteriocins.

The fact that there is some strain-dependent variation in immunity protein functionality (21, 22) suggests that the proteins may, however, interact indirectly with bacteriocins via cell components that vary somewhat between strains. The cell component could for instance be a receptor. The immunity proteins could then act by binding to the cytoplasmic side of the receptor and thereby block the ability of the receptor to interact with the bacteriocin in a fashion that is necessary for bacteriocin action. The recent structural determination of an immunity protein for a pediocin-like bacteriocin revealed in the C-terminal part of the protein a flexible loop that might interact with a bacteriocin receptor (25). A mechanism whereby the immunity protein functions by inactivating the bacteriocin receptor would not only require that the strain-dependent variation in the receptor be large enough to cause a sufficient variation in the binding of the various pediocin-like bacteriocins to the receptor (as discussed above for the permease), but also that it be large enough to cause a sufficient variation in the binding of the various immunity proteins to the receptor (so that each immunity protein only blocks at most a few receptor variants). These conditions are necessary to account for the results that reveal that (i) the immunity proteins show a high degree of specificity with respect to the bacteriocin they recognize and (ii) the various bacteriocins show different target cell specificities.

REFERENCES

1. Ennahar, S., Sashihara, T., Sonomoto, K., and Ishizaki, A. (2000) FEMS Microbiol. Lett. 200, 155–159
2. Moll, G. N., Konings, W. N., and Driessen, A. J. (1999) Arch. Microbiol. 174, 407–410
3. Aukrust, T. W., Brurberg, M. B., and Nes, I. F. (1995) FEMS Microbiol. Lett. 132, 187–191
4. Moll, G. N., Konings, W. N., and Driessen, A. J. (1999) Antonie van Leeuwenhoek 76, 189–196
5. Wang, Y., Henz, M. E., Gallagher, N. L., Chai, S., Gibbs, A. C., Yan, L. Z., Stiles, M. E., Wishart, D. S., and Vederas, J. J. (1999) Biochemistry 38, 15438–15447
6. Chen, Y., Ludescher, R. D., and Montville, T. J. (1997) Appl. Environ. Microbiol. 63, 4770–4777
7. Kazaz, M., Nissen-Meyer, J., and Finland, G. (2002) Microbiology 148, 2019–2027
8. Miller, K. W., Schamber, R., Osmanaagouli, O., and Ray, B. (1988) Appl. Environ. Microbiol. 64, 2957–2963
9. Aukrust, T. W., Brurberg, M. B., and Nes, I. F. (1995) FEMS Microbiol. Lett. 132, 187–191
10. Moll, G. N., Konings, W. N., and Driessen, A. J. (1999) Arch. Microbiol. 174, 407–410
11. Aymerich, T., Holo, H., Håvarstein, L. S., Hugas, M., Garriga, M., and Nes, I. F. (1999) Appl. Environ. Microbiol. 64, 3275–3281
12. Fimland, G., Blegsmø, O. R., Sletten, K., Jung, G., Nes, I. F., and Nissen-Meyer, J. (1996) Appl. Environ. Microbiol. 62, 3313–3318
13. Uteng, M., Hauge, H. H., Markwick, P. F., Finland, G., Mantzilas, D., Nissen-Meyer, J., and Mühlethaler, M. (2003) Biochemistry 42, 11417–11426
14. Fregeau Gallagher, N. L., Saier, M., Nienicura, W. P., Nakashima, T. T., Stiles, M. E., and Vederas, J. J. (1997) Biochemistry 36, 15062–15072
15. Wang, Y., Henz, M. E., Gallagher, N. L., Chai, S., Gibbs, A. C., Yan, L. Z., Stiles, M. E., Wishart, D. S., and Vederas, J. J. (1999) Biochemistry 38, 15438–15447
16. Aymerich, T., Holo, H., Finland, G., Hauge, H. H., and Nissen-Meyer, J. (2002) in Unmodified Peptide-Bacteriocins (Class II) Produced by Lactic Acid Bacteria (Dutton, J. D., Hazell, M. A., McCarthy, H. A. I., and Wax, R. G., eds) pp. 81–115, Marcel Dekker, Inc., New York
9250

Structure-Function Analysis of Pediocin-like Peptides

41. Brurberg, M. B., Nes, I. F., and Eijsink, V. G. (1997) _Mol. Microbiol._ 26, 347–369
42. Ramnath, M., Arous, S., Gravesen, A., Hastings, J. W., and Héchard, Y. (2004) _Microbiology_ 150, 2663–2668
43. Ramnath, M., Beukes, M., Tamura, K., and Hastings, J. W. (2000) _Appl. Environ. Microbiol._ 66, 3098–3101
44. Gravesen, A., Ramnath, M., Rechinger, K. B., Andersen, N., Jeansch, L., Héchard, Y., Hastings, J. W., and Knöchel, S. (2002) _Microbiology_ 148, 2361–2369
45. Dalet, K., Cenatiempo, Y., Cossart, P., and Héchard, Y. (2001) _Microbiology_ 147, 3263–3269
46. Héchard, Y., Pelletier, C., Cenatiempo, Y., and Frère, J. (2001) _Microbiology_ 147, 1575–1580
47. Saavedra, L., Minahk, C., de Ruiz Holgado, A. P., and Sesma, F. (2004) _Antimicrob. Agents Chemother._ 48, 2778–2781
The C-terminal Domain of Pediocin-like Antimicrobial Peptides (Class IIa Bacteriocins) Is Involved in Specific Recognition of the C-terminal Part of Cognate Immunity Proteins and in Determining the Antimicrobial Spectrum

Line Johnsen, Gunnar Fimland and Jon Nissen-Meyer

J. Biol. Chem. 2005, 280:9243-9250.
doi: 10.1074/jbc.M412712200 originally published online December 17, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M412712200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 18 of which can be accessed free at http://www.jbc.org/content/280/10/9243.full.html#ref-list-1