Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1705–1707
Bioorganic & Medicinal Chemistry Letters

Structure–activity relationship studies on quorum sensing $ComX_{RO\text{-}E\text{-}2}$ pheromone

Masahiro Okada,[a,*] Hisao Yamaguchi,[a] Isao Sato,[a] Soo Jeong Cho,[b,†] David Dubnau[b] and Youji Sakagami[a,*]

[a]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan*
[b]*Public Health Research Institute, 225 Warren Street Newark, NJ 07103, USA*

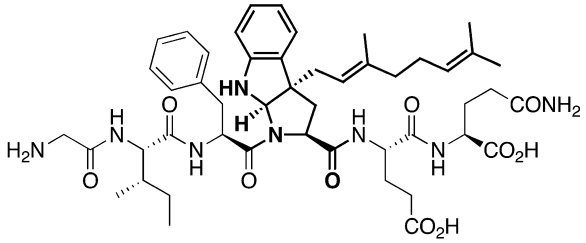
Received 19 September 2006; revised 5 December 2006; accepted 21 December 2006
Available online 23 December 2006

Abstract—The ComX pheromone is a posttranslationally modified oligopeptide that stimulates natural genetic competence in *Bacillus subtilis*. Various $ComX_{RO\text{-}E\text{-}2}$ analogs were synthesized and their biological activities were studied to investigate structure–activity relationships. These results showed that the minimal active unit was the tripeptide, [3–5]$ComX_{RO\text{-}E\text{-}2}$, and all residues except the modified tryptophan residue were replaceable by alanine without total loss of activity.

Bacteria secrete specific extracellular signaling molecules that increase in concentration with cell density. In a process known as quorum sensing, bacteria regulate their behavior in response to such molecules.[1] Quorum sensing attracts attention as a promising anti-pathogenic drug target rather than anti-bacterial, notably preventing the emergence of drug-resistant bacteria.[2] The ComX pheromone[3] is a signaling oligopeptide that stimulates natural genetic competence controlled by quorum sensing in *Bacillus subtilis*.[4] In the presence of the ComX pheromone, the membrane-located receptor histidine kinase ComP autophosphorylates and donates phosphate via a two-component system to activate competence gene expression.[5] Previous studies indicated that the ComX pheromone is posttranslationally modified by the addition of an isoprenoid to a tryptophan residue.[6,7] Posttranslational isoprenoidal modification on the cystein residue[8] is widely observed in many important proteins such as the Ras oncoproteins, which play a crucial role in human tumor, and the isoprenoidal modification is essential for the Ras functions.[9] Therefore, the post-translational isoprenoidal modification is an attractive target for cancer therapy, but on the tryptophan residue, including other amino acid residues except cystein, is unprecedented.

Recently the $ComX_{RO\text{-}E\text{-}2}$ pheromone **1** from *B. subtilis* strain RO-E-2[6] was shown to be a hexapeptide possessing a modified tryptophan residue with a geranyl group. This novel modification resulted in the formation of a tricyclic structure (Fig. 1).[10]

Since further studies are required to elucidate the details of the function through the interaction between the ComX pheromone and ComP, various $ComX_{RO\text{-}E\text{-}2}$



$ComX_{RO\text{-}E\text{-}2}$ pheromone **1**

Figure 1. Chemical structure of $ComX_{RO\text{-}E\text{-}2}$ pheromone. Bold Trp*(Ger) represents the modified tryptophan residue with a geranyl group in the $ComX_{RO\text{-}E\text{-}2}$ pheromone (bold lines).

Keywords: *Bacillus subtilis*; ComX; Posttranslational modification; Quorum sensing; Structure–activity relationship.
* Corresponding authors at present address: Graduate School of Science, Tohoku University, Aoba, Sendai 980-8578, Japan. Tel.: +81 22 795 6555; fax: +81 22 795 6557 (M.O.); e-mail: okada-org@mail.tains.tohoku.ac.jp
† Jinju National University, Department of Microbiological Engineering, 150 Chilamdong, Jinju, Gyonam 660-758, Republic of Korea.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.070

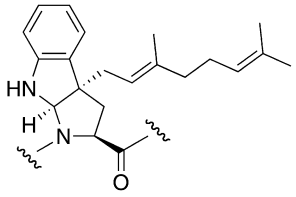
analogs were prepared using solid-phase peptide synthesis.[10–13] The biological activities of these peptides as well as of $ComX_{RO\text{-}E\text{-}2}$ pheromone were investigated using β-galactosidase assays.[6,10–12,14] These data were used to draw the dose response curves. The biological activities of these peptides were represented by two values both normalized to the activity of the $ComX_{RO\text{-}E\text{-}2}$ pheromone (Table 1). The first value presents the concentration of each peptide, needed to obtain the same β-galactosidase activity as that obtained with the $ComX_{RO\text{-}E\text{-}2}$ pheromone at EC_{50}. The second is the maximum activity as a percentage of that obtained with the $ComX_{RO\text{-}E\text{-}2}$ pheromone.

Although N-terminal truncated [2–6]$ComX_{RO\text{-}E\text{-}2}$ and [3–6]$ComX_{RO\text{-}E\text{-}2}$ showed significant biological activities as previously reported,[12] [4–6]$ComX_{RO\text{-}E\text{-}2}$ lost all activity (entry 4). The C-terminal glutamate residue was partially dispensable like the N-terminal glycine. In contrast, deletion of the glutamic acid and glutamate residues C-terminal to tryptophan dramatically decreased the activity (entries 5–8). These results indicated that for activity, either the modified tryptophan must reside at an internal position, or that contacts of the ComP receptor with the glutamic acid residue are important. A minimal active core within $ComX_{RO\text{-}E\text{-}2}$ appears to consist of the tripeptide, [3–5]$ComX_{RO\text{-}E\text{-}2}$ (entry 9). Elongation of $ComX_{RO\text{-}E\text{-}2}$ pheromone by the addition of alanine at either the N- or C-terminus did not affect the activity (entries 10 and 11). The roles of individual amino acid side chains were investigated by substitution of each residue except the modified tryptophan with alanine. Surprisingly, none were absolutely essential for biological activity, although alanine substitution of ^{3}phenylalanins and ^{6}glutamate decreased the activity substantially (entries 12–16).

Earlier work on the structure–activity relationships of tremerogen A-10,[15] which is the sex pheromone of basidiomycetous yeasts containing a C-terminal farnesyl modified cysteine methyl ester, revealed that removal of the C-terminus methyl ester or N-terminal residues decreased biological activity[16] in contrast to the present results with the $ComX_{RO\text{-}E\text{-}2}$ pheromone, which showed that only tripeptide, [3–5]$ComX_{RO\text{-}E\text{-}2}$, still had considerable activity. These results indicated that the pattern of specific interaction of the ComX pheromone with its receptor is unique and different from that of the S-isoprenoidal peptides. Furthermore, the precise structure of modified tryptophan residue was essential for the biological activity, because synthetic $ComX_{RO\text{-}E\text{-}2}$ peptides, containing a geranyltryptophan residue with a geranyl group replacing a tryptophanyl proton[10] or with additional stereoisomers at the modified tryptophan residue,[11,12] showed no biological activities. Although the farnesyl group on tremerogen A-10 was replaceable by lipophilic long chains,[16] the role of the geranyl moiety in biological activity and in determining pherotype specificity is not yet known. To investigate this question, we are synthesizing analogs of $ComX_{RO\text{-}E\text{-}2}$ with other side chains replacing the geranyl moiety.

In summary, various $ComX_{RO\text{-}E\text{-}2}$ analogs were synthesized and their biological activities were studied to investigate structure–activity relationships. These results showed that the minimal active unit was the tripeptide,

Table 1. Biological activities of $ComX_{RO\text{-}E\text{-}2}$ analogs

Trp*(Ger) ≡ 

Entry	Substance	Amino acid sequence	EC_{50}[a] (nM)	ED_{max}[b] (%)
1	$ComX_{RO\text{-}E\text{-}2}$	Gly-Ile-Phe-**Trp*(Ger)**-Glu-Gln	1	100
2	[2–6]$ComX_{RO\text{-}E\text{-}2}$	Ile-Phe-**Trp*(Ger)**-Glu-Gln	6	100
3	[3–6]$ComX_{RO\text{-}E\text{-}2}$	Phe-**Trp*(Ger)**-Glu-Gln	8	70
4	[4–6]$ComX_{RO\text{-}E\text{-}2}$	**Trp*(Ger)**-Glu-Gln	>300	15
5	[1–5]$ComX_{RO\text{-}E\text{-}2}$	Gly-Ile-Phe-**Trp*(Ger)**-Glu	20	80
6	[1–4]$ComX_{RO\text{-}E\text{-}2}$	Gly-Ile-Phe-**Trp*(Ger)**	130	60
7	[2–5]$ComX_{RO\text{-}E\text{-}2}$	Ile-Phe-**Trp*(Ger)**-Glu	20	80
8	[2–4]$ComX_{RO\text{-}E\text{-}2}$	Ile-Phe-**Trp*(Ger)**	160	55
9	[3–5]$ComX_{RO\text{-}E\text{-}2}$	Phe-**Trp*(Ger)**-Glu	20	60
10	Ala-$ComX_{RO\text{-}E\text{-}2}$	Ala-Gly-Ile-Phe-**Trp*(Ger)**-Glu-Gln	1	100
11	$ComX_{RO\text{-}E\text{-}2}$-Ala	Gly-Ile-Phe-**Trp*(Ger)**-Glu-Gln-Ala	7	100
12	[G1A]$ComX_{RO\text{-}E\text{-}2}$	Ala-Ile-Phe-**Trp*(Ger)**-Glu-Gln	3	100
13	[I2A]$ComX_{RO\text{-}E\text{-}2}$	Gly-Ala-Phe-**Trp*(Ger)**-Glu-Gln	10	95
14	[F3A]$ComX_{RO\text{-}E\text{-}2}$	Gly-Ile-Ala-**Trp*(Ger)**-Glu-Gln	20	90
15	[E5A]$ComX_{RO\text{-}E\text{-}2}$	Gly-Ile-Phe-**Trp*(Ger)**-Ala-Gln	3	100
16	[Q6A]$ComX_{RO\text{-}E\text{-}2}$	Gly-Ile-Phe-**Trp*(Ger)**-Glu-Ala	20	95

[a] Concentration of each peptide showing the same activity to the observed activity at EC_{50} of $ComX_{RO\text{-}E\text{-}2}$ pheromone.
[b] Ratio of maximum activity compared with $ComX_{RO\text{-}E\text{-}2}$ pheromone.

[3–5]Com$X_{RO\text{-}E\text{-}2}$, and all residues except the modified tryptophan residue were replaceable by alanine without total loss of activity.

Acknowledgments

The work done at Nagoya University was supported by Grant-in-Aid for COE (14COEA02) and Scientific Research (No. 18101009). The work done at PHRI was supported by US National Institutes of Health Grant GM57720.

References and notes

1. Recent reviews: (a) Lyon, G. J.; Muir, T. W. *Chem. Biol.* **2003**, *10*, 1007; (b) Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319; (c) Camilli, A.; Bassler, B. L. *Science* **2006**, *311*, 1113.
2. Dong, Y.-H.; Wang, L.-H.; Xu, J.-L.; Zhang, H.-B.; Zhang, X.-F.; Zhang, L.-H. *Nature* **2001**, *411*, 813.
3. Magnuson, R.; Solomon, J.; Grossman, A. D. *Cell* **1994**, *77*, 207.
4. (a) Weinrauch, Y.; Penchev, R.; Dubnau, E.; Smith, I.; Dubnau, D. *Genes Dev.* **1990**, *4*, 860; (b) Piazza, F.; Tortosa, P.; Dubnau, D. *J. Bacteriol.* **2001**, *181*, 4540.
5. Recent reviews: (a) Tortosa, P.; Dubnau, D. *Curr. Opin. Microbiol.* **1999**, *2*, 588; (b) Hamoen, L. W.; Venema, G.; Kuipers, O. P. *Microbiology* **2003**, *149*, 9.
6. Ansaldi, M.; Dubnau, D. *Mol. Microbiol.* **2002**, *44*, 1561.
7. Bacon Schneider, K.; Palmer, T. M.; Grossman, A. D. *J. Bacteriol.* **2002**, *184*, 410.
8. Clarke, S. *Annu. Rev. Biochem.* **1992**, *61*, 355.
9. Mazieres, J.; Pradines, A.; Favre, G. *Cancer Lett.* **2004**, *206*, 159.
10. Okada, M.; Sato, I.; Cho, S. J.; Iwata, H.; Nishio, T.; Dubnau, D.; Sakagami, Y. *Nat. Chem. Biol.* **2005**, *1*, 23.
11. Okada, M.; Sato, I.; Cho, S. J.; Suzuki, Y.; Ojika, M.; Dubnau, D.; Sakagami, Y. *Biosci. Biotech. Biochem.* **2004**, *68*, 2374.
12. Okada, M.; Yamaguchi, H.; Sato, I.; Dubnau, D.; Sakagami, Y. *Tetrahedron* **2006**, *62*, 8907.
13. Fmoc-protected modified tryptophan residue with a geranyl group was synthesized as previously reported,[10–12] and other residues were purchased from commercial sources (Watanabe chemical, Nova biochem). Peptide bond formation was accomplished with a peptide synthesizer except for the modified tryptophan coupling. After each crude peptide was purified by HPLC, the resulting peptides were confirmed by HRMS and MS/MS analyses. Each Com$X_{RO\text{-}E\text{-}2}$ peptide (about 1 mg) was obtained in 1–10% yield based on each resin.
14. Using the *B. subtilis* tester strain employed in the expression of an *srfA-lacZ* fusion, which responds to add Com$X_{RO\text{-}E\text{-}2}$ pheromone,[6] biological activity was investigated as previously reported.[6,10–12] Briefly, the strain was grown overnight and then diluted 100-fold. The culture was added to a sample solution and incubated at 37 °C for 5 h at 150 rpm, then β-galactosidase activity was measured at 420 nm with a standard method using *o*-nitrophenyl-β-D-galactopyranoside at 30 °C. Each analog was tested at concentrations up to 300 nM, because the response to the Com$X_{RO\text{-}E\text{-}2}$ pheromone and to analogs possessing the modified tryptophan residue achieved saturation at this concentration.
15. Sakagami, Y.; Yoshida, M.; Isogai, A.; Suzuki, A. *Science* **1981**, *212*, 1525.
16. Fujino, M.; Kitada, C.; Sakagami, Y.; Isogai, A.; Tamura, S.; Suzuki, A. *Naturwissenschaften* **1980**, *67*, 406.