Journal of Biosciences and Medicines, 2015, 3, 50-55
Published Online March 2015 in SciRes. http://www.scirp.org/journal/jbm
http://dx.doi.org/10.4236/jbm.2015.33008

The Two-Component Sensor Protein CovS Affects Penicilling Susceptibility by Modulation of Cell-Wall Synthesis in *Streptococcus pyogenes*

Masaaki Minami[1*], Syun Torii[1], Michio Ohta[2]

[1]Department of Bacteriology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan
[2]School of Nursing, Sugiyama Jyogakuen University, Nagoya, Japan
Email: [*]minami@med.nagoya-cu.ac.jp

Received December 2014

Abstract

In *Streptococcus pyogenes*, we have described the two-component signal transduction sensor and regulatory systems, CovR/S affect the antimicrobial susceptibility including penicillin G before. But the mechanism how two-component sensor protein CovS modulates penicillin G susceptibility has not been elucidated till date. This study aimed to determine how the CovS affected penicillin G susceptibility in *Streptococcus pyogenes* by northern blot analysis. At first, we investigated the *covS* mRNA expression under penicillin G induction. We found that the decrease of *covS* mRNA expression under Penicillin G stimulation. Next we investigated the expression of cell wall synthesis gene, *pbp2a* and *glmM* with use of *covS* knockout mutants from *emm*1 *Streptococcus pyogenes* strain 1529. We found that the cell-wall synthesis gene expression of the Δ*covS* mutant strain were lower than that of the wild-type strain. Furthermore the expression of *glmM* mRNA gene was lower than the expression of *pbp2a* mRNA gene in the Δ*covS* mutant strain. The *covS*-complemented strain almost restored the mRNA expression compared to Δ*covS* mutant strain. The two-component sensor protein CovS affects the susceptibility to penicillin G in *Streptococcus pyogenes* by modulation of cell-wall synthesis.

Keywords

Streptococcus Pyogenes, CovS, pbp2a, glmM

1. Introduction

Streptococcus pyogenes is a gram-positive bacterium that infects the upper respiratory tract, including the tonsils and pharynx, and is responsible for post-infectious diseases, such as rheumatic fever, glomerulonephritis, and streptococcal toxic shock-like syndrome [1]. Although the pathogenesis of *Streptococccus pyogenes* is unclear, many virulent proteins are considered to be causative factors.

[*]Corresponding author.

How to cite this paper: Minami, M., Torii, S. and Ohta, M. (2015) The Two-Component Sensor Protein CovS Affects Penicilling Susceptibility by Modulation of Cell-Wall Synthesis in *Streptococcus pyogenes*. *Journal of Biosciences and Medicines*, **3**, 50-55. http://dx.doi.org/10.4236/jbm.2015.33008

In the basic model of two-component systems, interaction of an appropriate extracellular stimulus with the sensor histidine kinase alters the phosphorylation state of its cytoplasmic domain [2]. The prototypic sensor protein has kinase and/or phosphatase activity for a cognate regulator protein; phosphorylation (or dephosphorylation) of the regulator controls its activity as a transcriptional activator or repressor for one or more target genes [3]. In many cases, signaling through a single two-component system results in a coordinated change in expression of multiple genes whose products play a role in adaptation to a particular environment [2]. Two-component systems CovR/S in *Streptococcus pyognes* has been shown to regulate expression of several virulence determinants, including the hyaluronic acid capsule, streptolysin S, and streptokinase [2] [3]. Microarray transcriptional profiling studies suggest that CovR/S regulates expression, directly or indirectly, of 15% of *Streptococcus pyogenes* genes [2] [3].

We described that CovR/S system may play a role in antibiotics susceptibility before [4]. However, the precise mechanism between CovS and penicillin G susceptibility has not yet been elucidated. Here, we focused on the role of cell-wall syntheses and evaluated whether CovS modulates cell-wall synthesis in *Streptococcus pyogenes.*

2. Materials and Methods

2.1. Bacterial Strains and Culture Condition

M1 serotype (*emm*1 genotype) *Streptococcus pyogenes* strains 1529 used in this study were clinical isolates from hospital patients in Japan with invasive *Streptococcus pyogenes* infections [4]. We also used *covS* (ΔcovS) knockout and *covS*-complemented (ΔcovS comp) mutants from *Streptococcus pyogenes* strains 1529 [4]. *Streptococcus pyogenes* was usually cultured in 5 mL of Brain-Heart Infusion Broth (Eiken Chemical Co., Tokyo, Japan) containing 0.3% yeast extract (Difco Laboratories, Detroit, MI, USA) (BHI-YE) for 18 - 24 h at 37˚C without agitation. The following antibiotic concentrations were used when appropriate: penicillin G (Sigma Chemical Co, St. Louis, MO, USA), 0.1 μg/mL, spectinomycin (Sigma Chemical Co), 100 μg/mL.

2.2. RNA Isolation and Northern Blot Analysis

Total RNA was extracted and purified as described previously [5] [6]. In brief, bacterial cells were cultured in 5 mL of BHI-YE. The cells were harvested at approximately 0.6 absorbance (log phase) at O.D.660. When induction study, the cells were cultured with penicillin G for further 15 minutes. Total RNA was extracted and purified with ISOGEN (Wako Pure Chemical Industries, Osaka, Japan). Approximately 2 μg of each of the total RNA preparation was electrophoresed on 1% agarose gel containing 1.1 M formaldehyde (Wako Pure Chemical Industries). RNAs were transferred onto a Hybond-N+ membrane (GE Healthcare, Waukesha, WI, USA). The DNA probes (*covS* (Spy0337)), *pbp2a* (Spy2059), *glmM* (Spy1038), and *rpsL* (Spy0271)) were amplified with oligonucleotide primers as follows: csrS-n4 (5'-ACAAGGCTCATTTGACCCAC-3') and csrS-c1 (5'-GCACTGCAGCTAACTCTCTTTAGACTGGG-3'); pbp2a-f1 (5'-AGCACTCAGGCATCTTACAT-3') and pbp2a-r1 (5'-TCCAAATAGATTGGGCTTTA-3'); glm-f1 (5'-CAGCACGTGATGTCTTTTTA-3') and glm-r1 (5'-TAATACGATCACCGTCAACA-3'); rpsL-f1(5'-GAATGTAGATGCCTACAATTAACCA-3') and rpsL-r1 (5'-TTTACGACTCATTTCTCTTTATCCC-3') [5]. These probes were ^{32}P-labeled using the random primer DNA labeling kit version 2 (Takara Bio, Ohtsu, Japan). The membranes were then autoradiographed and analyzed at room temperature with a bioimaging analyzer (BAS-1800II; Fujifilm, Tokyo, Japan). The express of *rpsL* mRNA was evaluated as internal control. The data from the bioimaging analyzer were calculated quantitatively.

2.3. Statistical Analysis

Statistical significance between the mean values was determined by one-way analysis of variance. A confidence interval with a p value of <0.05 was considered to be significant. The compared experiments were repeated a minimum of three times to improve the resulting data.

3. Result

3.1. Northern Blot Analysis Revealed That the Expression Level of *covS* mRNA Was Decreasing under PCG Induction in 1529 Wild-Type Strain

In our previous study, we suggested that CovS contributed to the effect of penicillin G treatment [4]. Hence, us-

ing northern blot analysis, we first analyzed whether penicillin G induced the expression of *covS*. As expected, after penicillin G treatment, the level of *covS* mRNA was increasing in comparison with the level without penicillin G induction (**Figure 1**).

3.2. Northern Blot Analysis Revealed That the Expression Level of *pbp2a* mRNA Was Decreasing in the 1529*covS* Mutant Strain

Next we evaluated the expression of *pbp2a* mRNAs among *Streptococcus pyogenes* 1529 Δ*covS* mutant strains. Thus, we have confirmed the change of *pbp2a* mRNA expression in both wild-type, 1529 Δ*covS* strain, and *covS*-complemented strains by northern blot analysis. **Figure 2** shows that the expression of *pbp2a* mRNA in the 1529 Δ*covS* mutant strain was lower than that in the wild-type strain.

3.3. Northern Blot Analysis Revealed That the Expression Level of *glmM* mRNA Was Decreasing in the 1529*covS* Mutant Strain

Furthermore, we evaluated the expression of *glmM* mRNAs among *Streptococcus pyogenes* 1529 Δ*covS* mutant strains. Thus, we have confirmed the change of *glmM* mRNA expression in both wild-type, 1529 Δ*covS* strain, and *covS*-complemented strains by northern blot analysis. **Figure 3** shows that the expression of *glmM* mRNA in the 1529 Δ*covS* mutant strain was lower than that in the wild-type strain.

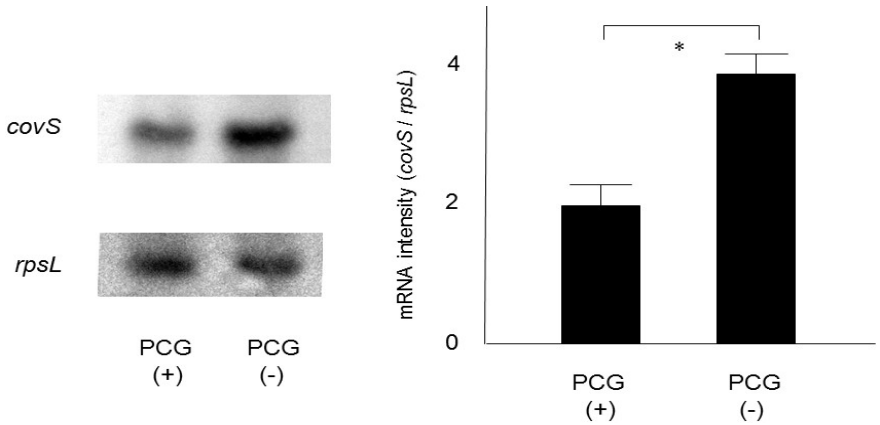


Figure 1. The expression of *covS* and *rpsL* mRNA under penicillin G induction by northern blot analysis. PCG: penicillin G. Asterisk indicates $p < 0.05$.

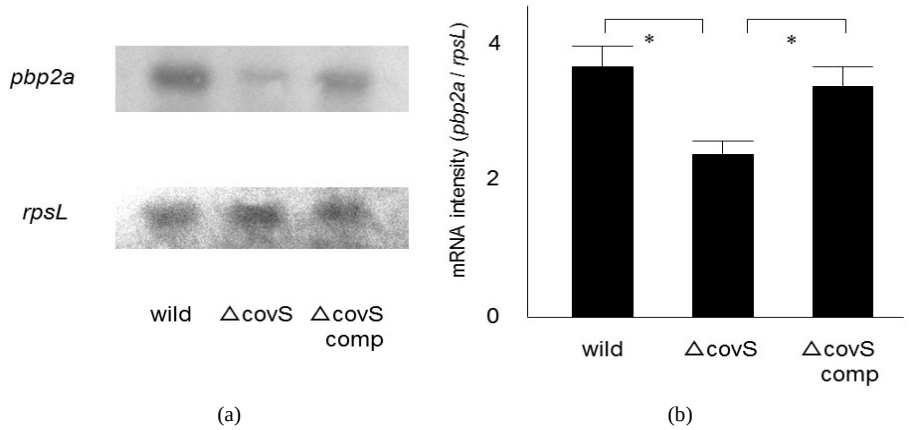
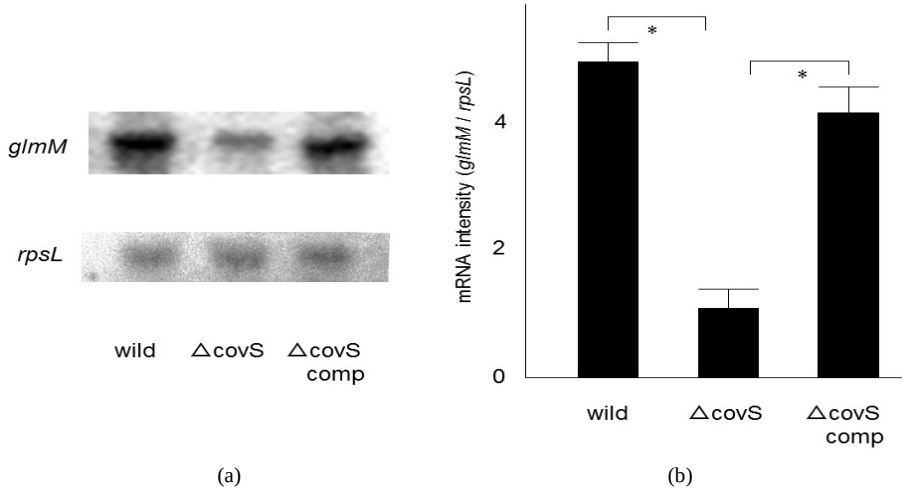


Figure 2. The expression of *pbp2a* and *rpsL* mRNA in 1529covS derived strains by northern blot analysis. Wild: 1529 wild-type, ΔcovS: 1529 *covS* knockout mutant, ΔcovS comp: 1529 *covS*-complemented mutant. Asterisks indicate $p < 0.05$.



(a) (b)

Figure 3. The expression of *glmM* and *rpsL* mRNA in 1529 covS derived strains by northern blot analysis. Wild: 1529 wild-type, ΔcovS: 1529 *covS* knockout mutant, ΔcovS comp: 1529 *covS*-complemented mutant. Asterisks indicate $p < 0.05$.

4. Discussion

In this study, we clarified that the mechanism between the *Streptococcus pyogenes* two component sensor protein CovS and penicillin G susceptibility. We have demonstrated that *covS*-inactivation in *Streptococcus pyogenes* was associated with increased penicillin G susceptibility before. Although this mechanism has been unclear, we have suggested two hypotheses from previous investigations [4]. One hypothesis is that CovS may affect the bacterial growth [4]. Previous report showed that a Δ*covS* mutant strain had lower growth ability than wild-type strain [7]. Antibiotic stress may result in the wideness of the growth differences between wild-type and *covS* mutant strains. Another hypothesis is that CovS may affect the function of PBP2a [4]. The *pbp2a* gene encodes transpeptidase which plays a role in cross linking of cell-wall [8]. Previous report represented that Δ*covR* mutant increased the expression of *pbp2a* mRNA compared to wild-type strain in microarray study [2]. CovS regulates the expression of CovR negatively [7]. The lack of *covS* may decrease the PBP2a activity via the uptake of *covR* expression and may result in the weakness of cell-wall structure [4]. We clarified this point in this study. Penicillin G decreased the expression of *covS* gene in 1529 wild-type strain. The Δ*covS* mutant strain had lowercell-wall synthesis gene *pbp2a* gene and *glmM* gene than the wild-type strain. Many factors influence antimicrobial susceptibility, and each factor is subjected to complex cross-talk regulation. Our results revealed part of the penicillin G susceptibility in *Streptococcus pyogens*.

Penicillin-binding proteins (PBPs) are membrane-bound D, D-peptidases that have evolved from serine proteases [9]. These enzymes catalyze the transpeptidation reaction that cross-links the peptidoglycan of the bacterial cell wall [8]. *β*-lactam antibiotics, which are substrate analogues, covalently bind to the PBP active site serineand inactivate PBPs at concentrations that are about the sameas the MICs [9]. *Streptococcus pyogenes* possesses five major PBPs [8]. The high-molecular-weight PBPs (mass > 60 kDa) are the critical antibiotic targets [9]. PBPs 1, 2, and 3, which have high affinity for most *β*-lactam antibiotics, are essential for cell growth and survival of susceptiblestrains [8]. Binding of *β*-lactams by these PBPs is lethal [8]. Previous study revealed that most of the radioactive penicillin was bound to PBP 2a/b both *in vivo* and *in vitro* and that the source of the amounts of radioactivity transferred to PBP3 was PBP 2 [8]. Although low-molecular-weight PBP 4 and 5 may be important in normal cell wall synthesis and participate to a limited extentin resistance, they are not considered a critical target and are dispensable [8]. Thus we focused on *pbp2a* gene expression in this investigation.

UDP-N-acetylglucosamine (UDP-GlcNAc) is an essential common precursor for synthesis of bacterial cell-wall peptidoglycan and outer membrane lipopolysaccharide [10] [11]. It is a directly glycosyl donor of linker unit and involved in the attachment ofgalactofuran and peptidoglycan [10] [11]. In bacteria, UDP-GlcNAc is synthesized from the glycolytic intermediate D-fructose-6-phosphate by four reactions catalyzed by three enzymes: glucosamine-6-phosphate synthase (GlmS), phosphogluco samine phosphogluco samin emutase (GlmM; EC 5.4.2.10) and the bifunctional enzyme glucosamine-1-phosphate acetyltransferase/N-acetylglucosamine-1-

phosphate uridyltransferase (GlmU) [10] [11]. Especially, GlmM catalyzes the inter conversion of glucosamine-6-phosphate to glucosamine-1-phosphate, an essential step in the biosynthetic pathway leading to the formation of the peptidoglycan precursor UDP-GlcNAc [10] [11]. A mutation in the *glmM* gene affects both peptidoglycan and lipopolysaccharide synthesis in *Escherichia coli* [10] [11]. Homologs of *glmM* in *Staphylococcus aureus* and *Streptococcus gordonii* are associated with sensitivity to antibiotics [10] [11]. Thus we supposed that the expression of *glmM* gene in *Streptococcus pyogenes* were also associated with penicillin G susceptibility in this study.

One mechanism for adaptation to changing environments is through two-component systems, a family of proteins that are widely distributed among many bacterial genera [2] [3]. Two-component systems allow sensing of specific environmental signals through a sensor histidine kinase that is usually associated with the cell membrane [2] [3]. Various antibiotic stresses have been reported to be recognized by other bacterial two-component systems. VraSR from *Staphylococcus aureus* is induced by bacitracin and vancomycin and also by other cell-wall antibiotics such as D-cycloserine [12]. A VraSR knockout strain shows a significant increase in sensitivity to the antibiotics it senses [12]. Although we demonstrated that two-component system CovR/S affected antibiotic susceptibility and that not only PBP2a but also GlmM play a role in penicillin Gsusceptibility via CovR/S system, the comparative investigation of other two-component systemsin *Streptococcus pyogenes* may be also necessary according to the antibiotic susceptibility pattern.

5. Conclusion

In summary, we clarified that the mechanism between the *Streptococcus pyogenes* two-component sensor protein CovS and penicillinG susceptibility by modulation of cell-wall synthesis. In particular, CovS may play an important role in the enzymatic activity of not only membrane-bound transpeptidase but also phosphoglucosaminemutase. Further investigations are needed to elucidate the mechanism of antimicrobial susceptibility in *Streptococcus pyogenes* via two-component signal transduction sensor and regular system pathways.

Acknowledgements

We thank Mr. Shoji Ishihara and Ms. Miwako Fujimura for special encouragement. We also thank the members of the department of bacteriology for their technical and material supports throughout this investigation. This study was supported by a Grant-in-Aid for Research from the Nagoya City University, Japan and by grants (numbers 23590889) from the Ministry of Education, Science, and Culture of Japan.

References

[1] Cunningham, M.W. (2000) Pathogenesis of Group A Streptococcal Infections. *Clinical Microbiology Review*, **13**, 470-511. http://dx.doi.org/10.1128/CMR.13.3.470-511.2000

[2] Graham, M.R., Smoot, L.M., Migliaccio, C.A., Virtaneva, K., Sturdevant, D.E., Porcella, S.F., *et al.* (2002) Virulence Control in Group A Streptococcus by a Two-Component Gene Regulatory System: Global Expression Profiling and *in Vivo* Infection Modelling. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 13855-13860. http://dx.doi.org/10.1073/pnas.202353699

[3] Shelburne 3rd, S.A., Sumby, P., Sitkiewicz, I., Granville, C., DeLeo, F.R. and Musser, J.M. (2005) Central Role of a Bacterial Two-Component Gene Regulatory System of Previously Unknown Function in Pathogen Persistence in Human Saliva. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 16037-16042. http://dx.doi.org/10.1073/pnas.0505839102

[4] Minami, M., Torii, S. and Ohta, M. (2014) CovS Modulates the Antimicrobial Susceptibility of *Streptococcus pyogenes*. *Scientific Journal of Microbiology*, **3**, 1-4.

[5] Minami, M., Kamimura, T., Isaka, M., Tatsuno, I., Ohta, M. and Hasegawa, T. (2010) Clindamycin-Induced CovS-Mediated Regulation of the Production of Virulent Exoproteins Streptolysin O, NAD Glycohydrolase, and Streptokinase in *Streptococcus pyogenes*. *Antimicrobial Agents and Chemotherapy*, **54**, 98-102. http://dx.doi.org/10.1128/AAC.00804-09

[6] Minami, M., Takase, H., Sakakibara, R., Imura, T., Morita, H., Kanemaki, N., *et al.* (2014) LicT Modulates Biofilm Formation of *Streptococcus pyogenes*. *Journal of Biosciences and Medicines*, in Press. http://dx.doi.org/10.4236/jbm.2014.29001

[7] Dalton, T.L. and Scott, J.R. (2004) CovS Inactivates CovR and Is Required for Growth under Conditions of General

Stress in *Streptococcus pyogenes. Journal of Bacteriology*, **186**, 3928-3937. http://dx.doi.org/10.1128/JB.186.12.3928-3937.2004

[8] Gutmann, L., Williamson, R. and Tomasz A. (1981) Physiological Properties of Penicillin-Binding Proteins in Group A Streptococci. *Antimicrobial Agents and Chemotherapy*, **19**, 872-880. http://dx.doi.org/10.1128/AAC.19.5.872

[9] Chambers, H.F. (1999) Penicillin-Binding Protein-Mediated Resistance in Pneumococci and Staphylococci. *The Journal of Infectious Diseases*, **179**, S353-S359. http://dx.doi.org/10.1086/513854

[10] Shimazu, K., Takahashi, Y., Uchikawa, Y., Shimazu, Y., Yajima, A., Takashima, E., *et al*. (2008) Identification of the *Streptococcus gordonii glmM* Gene Encoding Phosphoglucosamine Mutase and Its Role in Bacterial Cell Morphology, Biofilm Formation, and Sensitivity to Antibiotics. *FEMS Immunology Medical Microbiology*, **53**, 166-177.

[11] Glanzmann, P., Gustafson, J., Komatsuzawa, H., Ohta, K. and Berger Bächi, B. (1999) *glmM* Operon and Methicillin-Resistant *glmM* Suppressor Mutants in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, **43**, 240-245. http://dx.doi.org/10.1111/j.1574-695X.2008.00410.x

[12] Kuroda, M., Kuroda, H., Oshima, T., Takeuchi, F., Mori, H. and Hiramatsu, K. (2003) Two-Component System VraSR Positively Modulates the Regulation of Cell-Wall Biosynthesis Pathway in *Staphylococcus aureus*. *Molecular Microbiology*, **49**, 807-821. http://dx.doi.org/10.1046/j.1365-2958.2003.03599.x