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論文審査の結果の要旨

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学位論文題目

Studies on the pathogenic mechanisms of *Streptococcus intermedius*

*Streptococcus intermedius*の病原性制御機構の研究

審査結果の要旨

Streptococcus intermedius（SI）はアンギノーサス群連鎖球菌の一種であり、脳や肝臓等の深部臓器膿瘍や難治性の歯周病との関連性が指摘されている細菌である。

本菌はヒト細胞特異的な細胞溶解毒素インターメディリシン（ILY）を分泌し、これがヒトへの感染の必須な病原因子と考えられている。　従って、SI感染症を理解するためには、ILYの発現や活性調節の仕組みを解明することが重要と考えられる。

本研究は、*ily*遺伝子の発現を調節する様々な転写調節因子の解明やその調節機構、さらにはその転写調節シグナルとなる糖分子の獲得酵素の解明を行ったものである。

本研究では、*ily*遺伝子プロモーター領域のカタボライト抑制因子CcpAの結合配列に着目し、*ccpA*遺伝子破壊と相補により、*ily*遺伝子が資化糖で負の調節を受けることを明らかにした。さらに、ILY低産生性の歯垢株の遺伝子をランダム破壊することでLacRが*ily*遺伝子の主要な負の調節因子であることを示し、また重症SI感染症からの分離株ではこの*lacR*遺伝子に変異が起こったためにその機能が破綻してILY高産生株（強毒株）化し、感染症を引き起こした可能性も示した。また主論文では、4つのグリコシダーゼ活性を持ち、血清中の糖タンパク質の糖鎖を分解してLacRの不活性化とILY発現を誘導するガラクトースを生成するというSIの病原性発現に重要な役割を持つ新規グリコシダーゼMsgAを発見し、その性質を明らかにした。

以上のように学位申請者は、病原因子ILYの発現調節を介した*Streptococcus intermedius*の病原性制御機構の解明に見事に成功しており、この研究結果は本菌感染症の予防法や治療法の開発においても有用な知見となると考えられ，本論文は博士（工学）の学位授与に値するものと判定する。

なお本論文の審査には、友安俊文准教授の協力を得た。

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論　文　内　容　要　旨

報告番号	甲　先　第　**198**　号	氏　　名	今木　英統
学位論文題目	Studies on the pathogenic mechanisms of *Streptococcus intermedius* *Streptococcus intermedius*の病原性制御機構の研究		

内容要旨

Streptococcus intermedius belongs to the Anginosus group of streptococci (AGS) and is a human specific opportunistic pathogen. This pathogen causes purulent infections, including brain and liver abscesses. *S. intermedius* secretes a human-specific cytolysin, intermedilysin (ILY), which can bind specifically to the glycosylphosphatidylinositol-linked membrane protein, human CD59: a regulator of the terminal pathway of complement in humans. It is believed that ILY is a crucial virulence factor of this pathogen. Therefore, it is important to know the expressional control mechanisms of *ily* in order to understand the pathogenic mechanisms. To this end, we analyzed the nucleotide sequence of the *ily* promoter region and found a region that is highly homologous to the catabolite-repressible element (*cre*), a binding region for catabolite control protein A (CcpA). In addition, a considerable decrease of secreted ILY was observed when cells were grown in a culture medium containing high concentrations of glucose, one of utilizable carbohydrates. These data strongly suggested that CcpA could control the expression level of *ily*. Therefore, we disrupted the *ccpA* gene using an erythromycin cassette and found that this the *ccpA*-knockout strain did not induce catabolite repression of *ily* by utilizable carbohydrates. The strain also showed a prolonged lag phase and slower doubling time. In *cre* mutants, catabolite repression of *ily* was partially restored, and purified recombinant CcpA could bind to an oligo-DNA fragment containing the *cre* consensus sequence in the *ily* promoter region. We conclude from these data that *S. intermedius* can modulate *ily* expression through CcpA-mediated monitoring of the extracellular utilizable carbohydrate concentration.

It was reported that highly pathogenic strains could secret higher levels of ILY, although CcpA and *cre* mutations could not account for the difference between constitutively high ILY-producing strains and low ILY-producing (low pathogenic) strains. Therefore, we screened for a repressor of *ily* expression by using random gene disruption in a low ILY-producing strain (PC574), and succeeded in isolating three independent high ILY-producing colonies. These colonies had a plasmid insertion within a gene that has high homology to *lacR*. Validation of these observations was carried out by disruption of *lacR* in strain PC574 with an erythromycin-cassette. This led to higher hemolytic activity by increasing transcription of *ily* and higher cytotoxicity against the human liver hepatocellular carcinoma cell line (HepG2) cells compared to PC574. Adding lactose or galactose to the medium as a carbon source increased the amount of ILY secreted into the culture supernatant by PC574 cells. Furthermore, we examined *lacR* nucleotide sequences and the hemolytic activity of 50 strains isolated from clinical infections and 7 strains isolated from dental plaque. Of the 50 strains isolated from infections, 13 showed high ILY production, and 11 of these 13 strains had one or more amino acid exchange mutations and/or an insertion mutation

in LacR. Almost all mutations were associated with a marked decline in LacR function. These results strongly suggest that mutation of *lacR* is required for the overproduction of ILY, which is associated with an increase in the pathogenicity of *S. intermedius*.

Our observations strongly indicate that the amount and the type of sugar structures in the environment of the bacterial cell are important factors in the pathogenicity of *S. intermedius*. Interestingly, only *S. intermedius* could produce many glycosidase activities among the AGS strains. This strain shows β-D-galactosidase (β-Gal), β-D-fucosidase (β-Fuc), *N*-acetyl-β-D-glucosaminidase (β-GlcNAcase), *N*-acetyl-β-D-galactosaminidase (β-GalNAcase), and sialidase (NanA) activities. However, except for NanA, the enzyme(s) responsible for these glycosidase activities have not been identified yet. Therefore, we searched for the gene of each enzyme and found out a gene encoding a glycosidase in the *S. intermedius* chromosome with a large open reading frame (6.7 Kbp) in the *lac* operon. Bioinformatic analysis suggested that this gene encodes a novel glycosidase that has two domains (LacZ and GH20) and can exhibit β-Gal and *N*-acetyl-β-D-hexosaminidase activities, respectively. Therefore, we named this protein "multi-substrate glycosidase A" (MsgA). To test whether MsgA has these glycosidase activities, *msgA* in PC574 was disrupted with a spectinomycin-cassette. Interestingly, this mutant no longer showed cell- and supernatant-associated β-Gal, β-Fuc, β-GlcNAcase, and β-GalNAcase activities, and all phenotypes were complemented *in trans* with a plasmid carrying *msgA*. Purified MsgA had all of these four glycosidase activities and exhibited the lowest K_m with 4-methylumbelliferyl(4-MU)–linked *N*-acetyl-β-D-glucosaminide (the substrate for β-GlcNAcase) and the highest k_{cat} with 4-MU–linked β-D-galactopyranoside (the substrate for β-Gal). In addition, the purified LacZ domain of MsgA had β-Gal and β-Fuc activities and the GH20 domain exhibited both β-GlcNAcase and β-GalNAcase activities. The β-Gal and β-Fuc activities of MsgA are thermolabile, and the optimal temperature of the reaction was 40°C. Almost all enzymatic activities disappeared at 49°C. In contrast, β-GlcNAcase and β-GalNAcase activities were thermostable and the optimal temperatures were 58°C and 55°C, respectively. In addition we analyzed whether MsgA could remove the N-glycosylated sugar chains of human α_1-antitrypsin. Because removal of the sialic acid residues from the glycan branch end by NanA-treatment was necessary for degradation of glycan by MsgA on human α_1-antitrypsin, MsgA seems to have exoglycosidase activities.

Overall our results suggest that *S. intermedius* could obtain monosaccharides by MsgA and NanA from oligosaccharides, such as glycans as a nutrient for survival in a normal habitat. In addition, metabolic products of monosaccharides might regulate the expression level of *ily*, which through interactions with CcpA or LacR could control the pathogenicity of this pathogen.