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## 論 文 内 容 の 要 旨

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論文題名

Effect of agmatine on single species biofilm formation by *Porphyromonas gingivalis*(アグマチンが*Porphyromonas gingivalis*のバイオフィルム形成に及ぼす影響)

論文内容の要旨

**Introduction**

*Porphyromonas gingivalis* is considered to be a major etiologic factor of periodontal diseases and has a significant importance within biofilm. The pathogen expresses a number of virulence factors such as fimbriae, lipopolysaccharides and proteinases, among which a unique class of cysteine proteinases termed gingipains, composed of arginine-specific gingipain (Rgp) and lysine-specific gingipain (Kgp), are responsible for initiation and progression of marginal periodontitis. Furthermore, *P. gingivalis* is one of the few oral bacteria known to express peptidyl arginine deaminase, which converts arginine to citrulline, with the citrullination of periodontal tissue proteins thought to be related to development of disease. Arginine is also a precursor of another metabolic intermediate of polyamine and converted to agmatine by arginine decarboxylase (ADC), while agmatine is further converted to putrescine by agmatine deaminase. However, the roles of agmatine in oral biofilm ecology and periodontitis are unknown. In this study, we examined the influence of exogenous agmatine on *P. gingivalis*-single species biofilm development. Furthermore, we examined its effects on gingipain activities.

**Materials and methods**

To examine the effects of agmatine on biofilm formation by *P. gingivalis* cells, an *in vitro* biofilm formation assay was performed using 96-well polystyrene microtiter plates. *P. gingivalis* ( $1 \times 10^8$  CFU) was inoculated into chemically defined medium (CDM) with or without agmatine, then the plates were anaerobically incubated at 37°C for various time periods. Formed biofilms were stained with 1% crystal violet and amounts were measured at an absorbance of 550 nm, while change in optical density of the supernatant was also measured at 600 nm. For observations with confocal laser scanning microscopy (CLSM), *P. gingivalis* cells were stained with 5-(and-6)-carboxyfluorescein succinimidyl ester, followed by suspension in CDM with or without agmatine. Bacterial cells ( $1 \times 10^8$  CFU) were anaerobically cultured for 24 hours in saliva-coated chambers using a Culture Well™ chambered coverglass system and biofilm formation was analyzed with CLSM using Imaris Software Pro 7.0.1 (Biteplane AG). In order to investigate the effects of agmatine and putrescine on gingipain activity, a competitive inhibitory assay was performed.

Proteolytic activities were determined based on the fluorescence intensity of released 7-amino-4-methylcoumarin using an ARVO<sub>MX</sub>/light, and the activities of Rgp and Kgp were measured using the specific substrates Boc-Arg-MCA and Z-His-Glu-Lys-MCA, respectively.

## Results

Agmatine significantly stimulated biofilm formation, whereas it decreased the optical density of biofilm supernatant in time- and dose-dependent manners. CLSM observations revealed that agmatine clearly changed the microstructure of *P. gingivalis* biofilm to thick microcolonies that were dense and taller. Furthermore, addition of 10 mM agmatine induced clumpy detachment from biofilm. A competitive inhibitory assay indicated that 10 mM agmatine specifically inhibited Rgp activity by 98%, whereas the inhibitory effect of putrescine on Rgp activity was limited to 10%. In contrast, agmatine and putrescine exhibited the same inhibitory effect on Kgp activity.

## Discussion

Our results indicated a stimulatory effect of agmatine on *P. gingivalis* biofilm formation, as shown by crystal violet staining and confocal microscopic findings. Furthermore, it had a specific inhibitory effect on the Rgp activity of *P. gingivalis*, which is speculated to be based on it being structurally analogous to arginine. Agmatine likely competes with the arginine substrate side chain for uptake by the substrate active center of the Rgp enzyme. On the other hand, its non-specific low rate of inhibition of Kgp activity suggests that agmatine possesses a specific and essential role in Rgp activity. It was previously reported that a secreted arginine deaminase (ADI) produced by *Streptococcus intermedius* inhibited biofilm development by *P. gingivalis* by down-regulation of genes encoding *fimA* and *mfa1* fimbriae, both of which are required for proper biofilm development (Cugini *et al.*, 2013). Regardless of their origins, ADI and ADC in mixed species biofilms may act cooperatively as gatekeepers of biofilm virulence alteration by arginine metabolism.

## Conclusion

Intracellular and exogenous agmatine likely modulate biofilm formation by *P. gingivalis*. This arginine derivative was also found to inhibit Rgp activity. Together, the present results suggest that agmatine is involved in control of the ecology of oral biofilm formed by *P. gingivalis*.

論文審査の結果の要旨及び担当者

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| <b>論文審査の結果の要旨</b>  |                 |
| <p>本研究では、<i>Porphyromonas gingivalis</i> のバイオフィルム形成におけるアグマチンの役割について検討を加えた。その結果、アグマチンは同菌のバイオフィルムの肥厚ならびに剥離・拡散を促進した。さらに、バイオフィルム剥離・拡散に関与する Arg-gingipain 活性はアグマチンにより阻害され、アグマチンのバイオフィルム動態への制御的役割が示唆された。</p> <p>以上の研究成果はアグマチンによる <i>P. gingivalis</i> バイオフィルムの制御機構の一端を明らかにするものであり、博士（歯学）の学位論文に値するものと認める。</p> |                 |