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The Effects of Cyclodextrins on Autoinducer Activities of Quorum Sensing in *Pseudomonas aeruginosa*

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Abstract

Inclusion complex formation between cyclodextrin and autoinducer of gram negative bacteria in aqueous solution was investigated by 1D ^{1}H-NMR and ROESY spectra. An inhibition effect was observed on autoinducer activities of quorum sensing in *Pseudomonas aeruginosa* by adding cyclodextrins to the bacterial culture medium.

Introduction

Quorum sensing is one of the cell-cell communication mechanisms depending on cell population density in gram-negative bacteria. [1] Several kinds of *N*-acyl-l-homoserine lactones have been identified as signal compounds involved in this mechanism and called autoinducers (AIs). AIs are produced in bacteria, diffused outside and inside of bacteria and regulate expression of genes responsible for bioluminescence, secretion of virulence factors, expression of pathogenicities and forming biofilm, respectively. It is very important to develop the control methods of quorum sensing not only for the microbiology but also for the medicine, the pharmaceutics, the environmental science, the chemical engineering, etc. As cyclodextrins (CDs) are well known to make a complex with a wide variety of organic compounds, it is expected to form a complex between CD and AI in the bacterial culture medium and show the effect on AI activities of quorum sensing. The purpose of this study was to investigate the control methods of quorum sensing in gram negative bacteria by adding CDs into the bacterial culture medium.

Experimental

α-, β-, γ-, DM-β- and TM-β-CD from Nihon Shokuhin Kako Co., Ltd. were used without further purification.

For NMR measurements to determine the complex formation between CDs and AIs in aqueous solution, chemically synthesized C4 (Figure 1) [2], one of the AIs of *P. aeruginosa*, were used. 1D ^{1}H-NMR and ROESY experiments were performed using JEOL JNM-LA500 [3].

For determination of effects of CDs on AI activities, 10 mM CDs or glucose were added in the bacterial culture medium. We monitored activations of the transcription of the *rhlA-lacZ* genes in *Pseudomonas aeruginosa* PAO-1(pβ02) at the early stationary phase of growth. AI activities were determined as β-galactosidase activities by the Miller's standard procedure [2, 4].

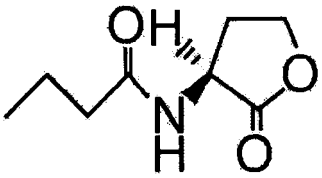

Figure 1. Structure of C4.

Results

1D ^{1}H-NMR spectra of α- or β-CD and C4 mixture showed upfield shifts of H(3) and H(5) protons of α- or β-CD (Figure 2) and downfield shifts of acyl protons of C4. ROESY spectrum of α- or β-CD and C4 mixture showed cross-peaks between H(3) and H(5) protons of α- or β-CD and acyl protons of C4 (Figure 3). In the case of using γ-CD, no induced chemical shift changes on the 1D ^{1}H-NMR spectra and no cross-peaks between protons of γ-CD and C4 on ROESY spectrum were observed. Bioassays using *P. aeruginosa* PAO-1(pβ02) revealed that AI activities were decreased by adding α-, β-, DM-β- or TM-β-CD into the culture medium. On the other hand, no effect was observed in the case of adding γ-CD or glucose. (Table 1) Every compound gave no effect on the growth of bacteria.

Discussion

The induced chemical shift changes on the 1D ^{1}H-NMR spectra and cross-peaks on the ROESY spectrum strongly

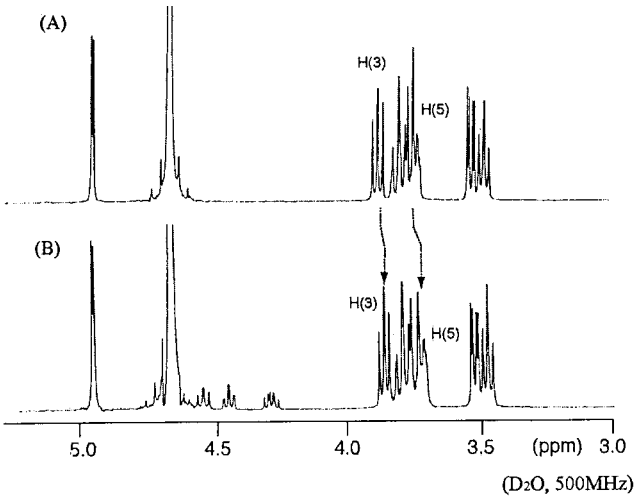

Figure 2. ^{1}H-NMR spectra of (A) α-CD alone and (B) α-CD–C4 mixture.

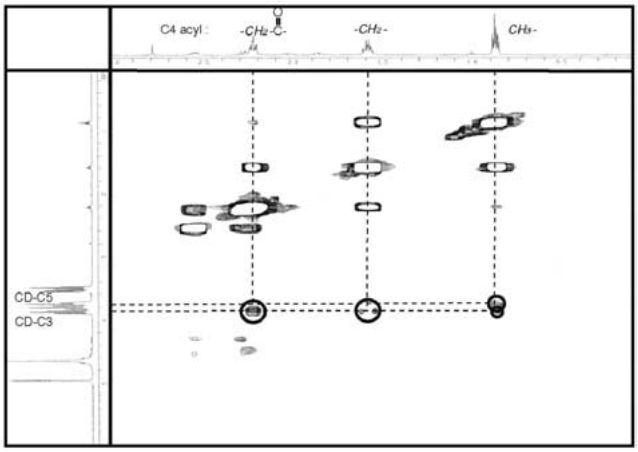

Figure 3. ROESY spectrum of α-CD–C4 mixture.

Table 1. β-galactosidase activities* as autoinducing activities

Control	+α-CD	+β-CD	+DM-β-CD	+TM-β-CD	+γ-CD	+glucose
1	0.21	0.26	0.31	0.29	0.98	0.99

* Miller units relative to control.

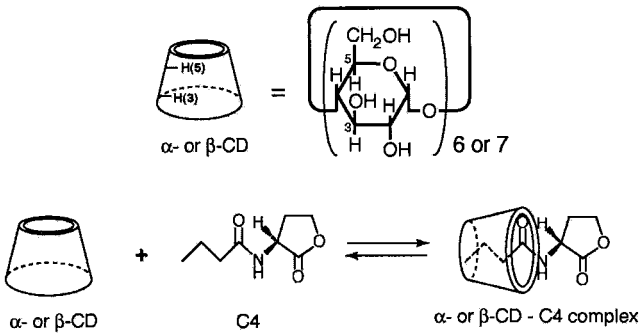

Figure 4. α- or β-cyclodextrin – C4 complex.

indicated that α- and β-CD bound a part of acyl side chain of chemically synthesized C4 (Figure 4). Because of the large cavity, γ-CD could not form an inclusion complex with C4. The dissociation constants (Kd) of the inclusion complex were calculated using the chemical shift changes of the acyl proton resonance of C4 induced by the added CD using the similar procedures as already reported [5]. Kd values for complexation were estimated to be 5.3×10^{-2} M for α-CD – C4 complex and 4.2×10^{-2} M for β-CD – C4 complex.

The bioassay results suggested that α- and β-CD could also form a complex with the natural AI produced by *P. aeruginosa* in the culture medium. Since the concentration of free AIs in the culture medium might be decreased by complexation with α- or β-CD, AI activities of quorum sensing in *P. aeruginosa* were seemed to be decreased. As DM-β-CD and TM-β-CD showed the similar effects as shown by β-CD, they could also form a complex with AI. On the other hand, γ-CD showed no effect on AI activity. γ-CD could not form a complex with C4 because of the size of the cavity as indicated also by NMR studies. Glucose could not also form a complex with C4 and showed no effect on AI activity.

As every additives showed no effect on the growth of bacteria, it seemed that they acted as host compounds for AI and inhibitors of AI activity.

In conclusion, α-, β-, DM-β- and TM-β-CD could form a complex with AI in the culture medium and showed decreasing effects on AI activities. This is the first approach to control AI activities and quorum sensing in gram-negative bacteria by adding host compounds of AIs. Of course, native CD has no specific binding ability to AI and there are several kinds of other organic compounds which can be bound to CD in the culture medium, huge amount of CD is needed to form a complex with AI and show the decreasing effect on AI activity compared with the concentration of natural AI which is nM $\sim$ μM level. Synthesis and development of modified CDs having specific binding ability to AI are now in progress.

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