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Solid-state NMR analysis of calcium and D-mannose binding of BMY-28864, a water-soluble analogue of pradimicin A

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ABSTRACT

Pradimicin A (PRM-A) is a unique antibiotic with a lectin-like ability to recognize D-mannopyranosides (Man) in the presence of Ca^{2+} ion. BMY-28864 (**1**) is a water-soluble analogue of PRM-A, which has been extensively used for studies on the mode of Man recognition and antifungal action of pradimicins. Although it has been assumed that PRM-A and **1** bind Man in a similar fashion, direct experimental evidence has yet to be provided. In this report, we compared Ca^{2+} and Man binding of **1** with that of PRM-A through two solid-state NMR experiments. The solid-state ^{113}Cd NMR analysis using $^{113}Cd^{2+}$ ion as a surrogate for Ca^{2+} ion suggested the similarity in Ca^{2+} coordination of PRM-A and **1**. The dipolar assisted rotational resonance (DARR) analysis using ^{13}C-labeled **1** clearly showed that **1** as well as PRM-A binds Man near its carboxyl group. These results collectively indicate that the mode of binding of Ca^{2+} ion and Man is nearly identical between PRM-A and **1**.

Pradimicin A (PRM-A, Fig. 1) and its congeners are the only family of natural antibiotics with a lectin-like ability to recognize D-mannopyranosides (Man) in the presence of Ca^{2+} ion.[1,2] In recent years, they have been attracting much attention as conceptually novel drug candidates for human immunodeficiency virus (HIV).[3,4] PRM-A has been shown to block the virus entry to the host cells and force the virus to progressively delete the envelope glycans which shield against the host immune system. This dual mode of antiviral action is ascribed to its specific binding to the high-mannose-type glycans on the viral envelope.

Despite significance in terms of scientific interest as well as therapeutic potential, the molecular basis of Man recognition by pradimicins remains to be clarified. The essence of the problem lies in their aggregate-forming propensity and complicated three-component equilibrium in solution, which have hampered conventional X-ray crystallographic and solution NMR analyses. Under these circumstances, we have recently developed a new strategy to analyze the ternary complex of PRM-A with Ca^{2+} ion and Man in the solid state.[5,6] The key of our analytical strategy is the use of the aggregate of the ternary complex of PRM-A with Ca^{2+} ion and methyl α-D-mannopyranoside (Man-OMe), which enabled us to analyze Ca^{2+} and Man binding of PRM-A by solid-state NMR spectroscopy. Our studies revealed that PRM-A binds Man in a Ca^{2+}-mediated manner through its carboxyl group, and the cavity consisting of D-alanine moiety and ABC rings constitutes a Man binding site (Fig. 2). These results suggest that D-alanine moiety plays pivotal roles in both Ca^{2+} and Man binding of PRM-A.

In fact, these results are in accordance with the previous structure–activity relationship studies of PRM-A. Replacement of the D-alanine moiety with L-alanine or most of other D-amino acids significantly diminished antifungal activity, which is correlated with Man binding ability of pradimicins.[7] The notable exceptions were D-serine analogues (**1**, **2**), which show significant antifungal activity comparable to PRM-A (Fig. 1).[8] Although additional *N*-methyl group of BMY-28864 (**1**) does not impact the antifungal activity, water solubility of **1** is significantly higher than those of PRM-A and **2** (0.02 mg/mL for PRM-A, >20.0 mg/mL for **1**, 0.26 mg/mL for **2** in phosphate buffered saline, pH 7.2).[8,9] These features have led **1** to be extensively used for studies on the mode of Man recognition and antifungal action of pradimicins.[2,10] Although it has been assumed a priori that PRM-A and **1** bind Man in a similar fashion, the emerging importance of D-alanine moiety of PRM-A requires direct experimental validation of this hypothesis. To clarify this issue, we compared Ca^{2+} and Man binding of **1** with that of PRM-A through two solid-state NMR experiments.

In the previous study, we investigated the Ca^{2+} binding property of PRM-A by employing solid-state ^{113}Cd NMR spectroscopy.[5] ^{113}Cd NMR spectroscopy has proven to be an excellent technique for analysis of the Ca^{2+} environments present in biological systems,

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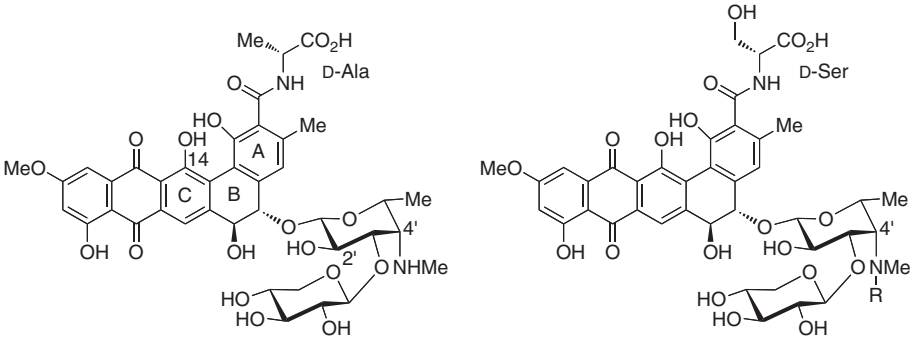


Figure 1. Structures of pradimicin A (PRM-A) and its serine analogues (**1**, **2**).

because $^{113}Cd^{2+}$ ion has an ionic radius similar to that of Ca^{2+} ion and favorable characteristics for NMR analysis such as a spin of 1/2, reasonable sensitivity, and a broad chemical shift window (>800 ppm).[11,12] $^{113}Cd^{2+}$ ion was confirmed to serve as a good surrogate for Ca^{2+} ion in Man binding of PRM-A, and successfully form a ternary complex with PRM-A and Man-OMe.[5] The solid aggregate of the complex exhibited a sharp signal at $\delta = -135$ ppm in the cross-polarization/magic angle spinning (CP/MAS) ^{113}Cd NMR spectrum, reflecting coordination of more than six oxygen ligands to $^{113}Cd^{2+}$ ion. Using this characteristic ^{113}Cd signal as a guide, we initially investigated Ca^{2+} binding of **1** by solid-state ^{113}Cd NMR spectroscopy.

BMY-28864 (**1**) was prepared according to the procedure reported by Oki et al. using *Actinomodura* sp. TP-A0019.[8,13] Although **1** formed little aggregates as a binary complex with $^{113}Cd^{2+}$ ion due to its high water solubility, the solid aggregate of the ternary complex consisting with $^{113}Cd^{2+}$ ion and Man-OMe was effectively obtained.[14] Comparison of the CP/MAS ^{113}Cd NMR spectra of the ternary complexes of PRM-A[5] and **1** is shown in Figure 3. The latter complex exhibited a sharper signal compared to the former. The narrower line width of the signal probably reflects the higher structural homogeneity of the ternary complex of **1**. The ^{113}Cd signal upfiled of $\delta = -100$ ppm suggests that $^{113}Cd^{2+}$ ion in the ternary complex of **1** as well as PRM-A is coordinated with more than six oxygen ligands.[11,12] Since ^{113}Cd nucleus has a broad range of chemical shifts (>800 ppm) that is sensitive to the nature, number, and geometry of ligands coordinated to $^{113}Cd^{2+}$ ion, it is reasonable to assume that the slightly downfield signal of **1** ($\delta = -129$ ppm) compared to that of PRM-A ($\delta = -135$ ppm) is ascribed to a subtle difference in the $^{113}Cd^{2+}$ environment in the ternary complexes. The realistic implication of this result is that the Ca^{2+} coordination in the ternary complex of **1** is almost similar to that of PRM-A.

Investigation of Man binding of PRM-A has been performed by two-dimensional dipolar assisted rotational resonance (2D-DARR)[15,16] using ^{13}C-enriched PRM-As.[5,6] DARR, also known as RF assisted diffusion (RAD),[17] is a technique of solid-state NMR spectroscopy to detect weak ^{13}C–^{13}C couplings in the presence of strong couplings derived from directly bound carbons. In 2D-DARR spectra, dipolar interactions between ^{13}C nuclei that are located within 6 Å can be detected as cross peaks. In the DARR experiments using the solid aggregates of the ternary complexes of ^{13}C-labeled PRM-As with Ca^{2+} ion and [$^{13}C_6$]Man-OMe, strong intermolecular cross peaks were observed between the ^{13}C signals for the D-alanine moiety of PRM-A and those for Man-OMe whereas the ^{13}C signal for the *N*-methyl group at position 4′ of PRM-A gave no intermolecular cross peak.[5,6] These observations clearly indicated that PRM-A binds Man in its proximity to the D-alanine moiety. In order to substantiate the specific close contact of Man with D-

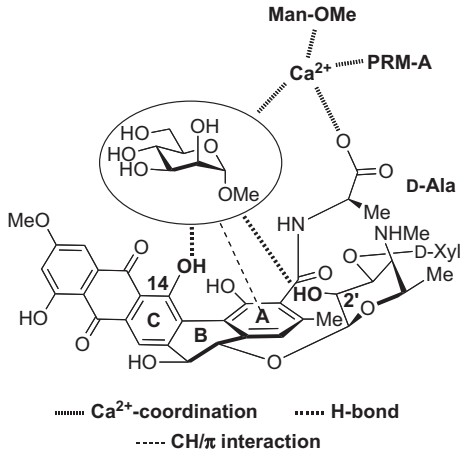


Figure 2. Putative binding model of PRM-A with Man-OMe.[6]

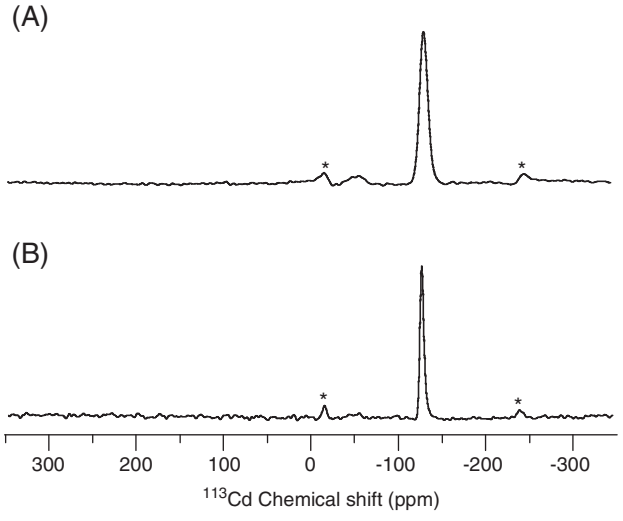
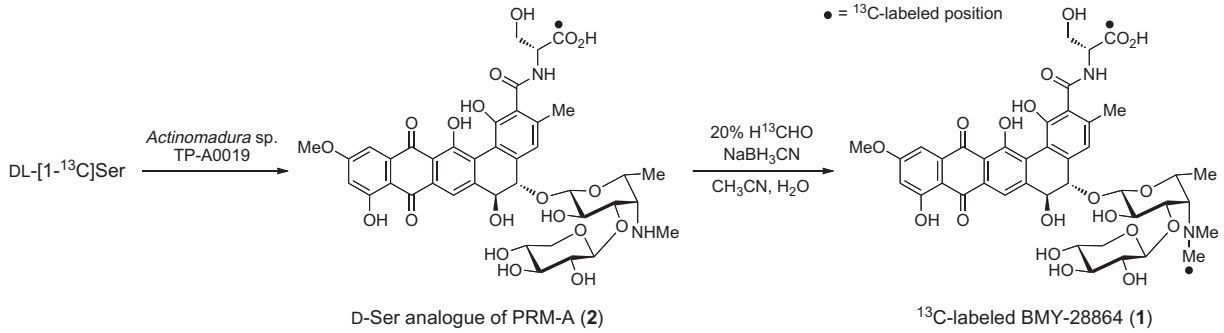


Figure 3. Solid-state CP/MAS ^{113}Cd NMR spectra of the ternary complexes of (A) PRM-A[5] and (B) **1** with $^{113}Cd^{2+}$ ion and Man-OMe. One equivalent of $^{113}CdCl_2$ and 250 equiv of Man-OMe were used for complete formation of the ternary complexes with minimizing non-specific binding of $^{113}Cd^{2+}$ ion to the aggregates.[5] Chemical shifts were calibrated in ppm relative to external solid $Cd(ClO_4)_2{\cdot}6H_2O$. The signals with an asterisk are the spinning side bands of the ^{113}Cd signals.



Scheme 1. Preparation of ^{13}C-labeled **1**.

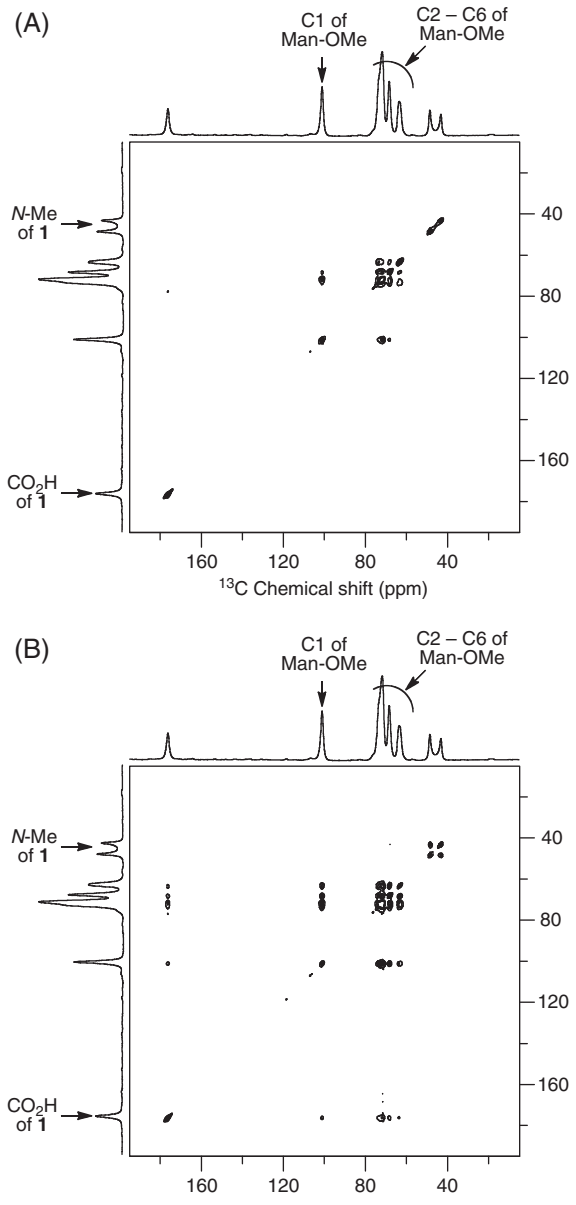


Figure 4. 2D-DARR spectra of the ternary complex of ^{13}C-labeled **1** with Ca^{2+} ion and [$^{13}C_6$]Man-OMe at the mixing times of (A) 20 ms and (B) 500 ms. Ten equivalent of $CaCl_2$ and 25 equiv of [$^{13}C_6$]Man-OMe were used for complete formation of the ternary complex with minimizing non-specific binding of [$^{13}C_6$]Man-OMe to the aggregates.[6] Chemical shifts were calibrated in ppm relative to TMS by taking the ^{13}C chemical shift for the methine ^{13}C of solid adamantane (δ = 29.5 ppm) as an external reference standard.

serine moiety of **1**, we planned to conduct DARR experiments using ^{13}C-labeled **1**.

To facilitate the DARR analysis, ^{13}C-labeling of the D-serine carboxyl group and *N*-methyl group of **1** was carried out (Scheme 1). Since a negligible amount of the D-serine analogue of PRM-A (**2**) is naturally produced by *Actinomodura* sp. TP-A0019, ^{13}C-labeling of the D-serine carboxyl group was easily accomplished by feeding of DL-[1-^{13}C]serine. The $^{13}CH_3$ group was then successfully introduced to the amine group at position 4′ by reductive methylation using H^{13}CHO (62%).[5] The solution ^{13}C NMR spectrum of resulting ^{13}C-labeled **1** confirmed a high level of ^{13}C-enrichment of the targeted two carbons (Supplementary data). Figure 4 shows the 2D-DARR spectra of the ternary complex of ^{13}C-labeled **1** with Ca^{2+} ion and [$^{13}C_6$]Man-OMe at mixing times of 20 and 500 ms. Whereas only intramolecular cross peaks were observed at the mixing time of 20 ms (Fig. 4A), significant intermolecular cross peaks between the ^{13}C signal for the D-serine carboxyl group (δ = 176.3 ppm) and those for Man-OMe (δ = 63.5, 68.4, 71–75, 101.2 ppm) were detected at the mixing time of 500 ms (Fig. 4B). On the other hand, no intermolecular cross peak was detectable for the $^{13}CH_3$ group (δ = 43.2, 48.6 ppm), which appeared as two signals probably because of slow inversion at the asymmetric nitrogen center. These observations indicate that **1** as well as PRM-A binds Man near the carboxyl group.

In summary, we compared Ca^{2+} and Man binding of BMY-28864 (**1**) with that of PRM-A through solid-state ^{113}Cd NMR and DARR experiments. The results suggest that PRM-A and **1** are almost the same in terms of Ca^{2+} coordination and Man binding site. This is the first direct experimental evidence that PRM-A and **1** bind Man in a similar fashion. Further investigations to understand why only pradimicins with D-alanine and D-serine moieties can bind Man are currently underway.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.106.

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