Pradimicin A, a D-mannose-binding antibiotic, binds pyranosides of L-fucose and L-galactose in a calcium-sensitive manner

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Abstract

Pradimicin A (PRM-A) is a unique antibiotic with a lectin-like ability to bind D-mannose (D-Man) in the presence of Ca^{2+} ion. Although accumulated evidences suggest that PRM-A recognizes the 2-, 3-, and 4-hydroxyl groups of D-Man, BMY-28864, an artificial PRM-A derivative, was shown not to bind L-fucose (L-Fuc) and L-galactose (L-Gal), both of which share the characteristic array of the three hydroxyl groups with D-Man. To obtain a plausible explanation for this inconsistency, we performed co-precipitation experiments of PRM-A with L-Fuc, L-Gal, and their methyl pyranosides (L-Fuc-OMe, L-Gal-OMe) by taking advantage of aggregate-forming propensity of the binary $[\text{PRM-A}/\text{Ca}^{2+}]$ complex. While L-Fuc and L-Gal were hardly incorporated into the aggregate, L-Fuc-OMe and L-Gal-OMe were found to exhibit significant binding to PRM-A. However, increased Ca^{2+} concentration abolished this binding, raising the possibility that poor binding of L-Fuc and L-Gal to PRM-A is attributed to their chelation with Ca^{2+} ion. This possibility was partly supported by $^1\text{H-NMR}$ analysis that detected interaction of L-Fuc and L-Gal with Ca^{2+} ion in aqueous solution. These results collectively indicate that PRM-A binds pyranosides of L-Fuc and L-Gal when Ca^{2+} concentration is not excessive to trap these sugars by chelation but sufficient to form the $[\text{PRM-A}/\text{Ca}^{2+}]$ complex.

Key words: antibiotic; carbohydrate recognition; lectin; natural product; pradimicin

Pradimicins (PRMs) and benanomycins comprise a unique family of antibiotics with a lectin-like ability to bind D-mannose (D-Man) in the presence of Ca^{2+} ion.¹⁻³ They have been attracting a great deal of attention as the only class of non-peptidic small molecules that can capture D-Man under physiologically relevant conditions. Coupled with the emerging pathological significance of carbohydrates, interests in their therapeutic potential have also been intensified in recent years. PRMs have been shown to exhibit antifungal and anti-HIV activities by binding to cell-wall mannans of microorganisms and high mannose-type glycans on the viral envelope of HIV, respectively.^{4,5} There exists no chemotherapeutics that directly target pathologically important glycans, underscoring their potential as promising drug candidates for a first-in-class approach to the infectious diseases.^{6,7}

Since the discovery that PRMs serve as lectin mimics, efforts have been made to understand the molecular basis of carbohydrate recognition by PRMs. UV-visible spectroscopic analysis using BMY-28864 (Fig. 1), an artificial PRM derivative with improved water solubility,⁸ revealed that PRMs and Ca^{2+} ion form the binary [PRM/ Ca^{2+}] complexes, which bind D-Man but not L-mannose, 2-, 3-, and 4-epimers of D-Man (D-glucose, D-altrose, and D-talose, respectively), D-mannosamine, and *N*-acetyl-D-mannosamine.^{9,10} This sugar-binding profile led to the assumption that PRMs recognize the 2-, 3-, and 4-hydroxyl groups of D-Man.

This assumption is supported by our solid-state NMR analysis using PRM-A (Fig. 1),² an original member of the PRM family.¹¹⁻¹³ Taking advantage of aggregate-forming propensity of the [PRM-A/ Ca^{2+}] complex, we prepared the solid

aggregate composed of the ternary complex of PRM-A with Ca^{2+} ion and methyl α -D-mannopyranoside (α -D-Man-OMe). Evaluation of interatomic distances between PRM-A and α -D-Man-OMe revealed that the C2–C4 region of D-Man is in close contact with PRM-A, while the C1 and C6 positions of D-Man are relatively distant in the [PRM-A/ Ca^{2+} / α -D-Man-OMe] complex. Based on the distance information, we proposed a binding model, in which the 2-, 3-, and 4-hydroxyl groups of D-Man form hydrogen bonds with PRM-A (Fig. 2). The importance of these hydroxyl groups was further confirmed by co-precipitation experiments using deoxy derivatives of α -D-Man-OMe. While α -D-Man-OMe and its 6-deoxy derivative were co-precipitated with the [PRM-A/ Ca^{2+}] complex, the 2-, 3-, and 4-deoxy ones were hardly incorporated into the aggregates. These combined results strongly support the conclusion that PRM-A binds D-Man by recognizing the spatial array of its 2-, 3-, and 4-hydroxyl groups.

This binding model well explains the fact that BMY-28864 also accommodates D-arabinose (D-Ara), D-fructose, and D-lyxose.¹⁰ These sugars have an arrangement similar to the 2-, 3-, and 4-hydroxyl groups of D-Man (Fig. 3), and are thus expected to bind to PRMs in the same manner as D-Man. However, the binding model provides no plausible explanation concerning the reason why BMY-28864 was insensitive to L-fucose (L-Fuc) and L-galactose (L-Gal),¹⁰ which can also mimic the array of the 2-, 3-, and 4-hydroxyl groups of D-Man (Fig. 3). In this study, we performed the co-precipitation experiments of PRM-A with L-Fuc, L-Gal, and their methyl pyranosides to assess the origin of poor binding of L-Fuc and L-Gal to PRMs.

In our previous studies,^{12,13} co-precipitation of PRM-A with sugars was conducted at pH 4.5 for effective aggregate formation of the [PRM-A/Ca²⁺] complex. Specific binding of sugars was quantified as molar ratio of sugars relative to PRM-A in the washed aggregates, which can be calculated by ¹H-NMR on the basis of integration values of PRM-A and sugars after dissociation of the aggregates by acid treatment. However, this procedure, though applicable to non-reducing sugars, was found to be inadequate for evaluating specific binding of reducing sugars. For example, when a co-precipitation experiment was performed using D-glucose (D-Glc), which is insensitive to PRMs,¹⁰ a significant amount of D-Glc was detected in the aggregate (D-Glc/PRM-A = 0.57). One possible reason is reversible adduct formation derived from reaction of the aldehyde group of D-Glc with the amino group at C4' of PRM-A under weak acidic conditions (around pH 4.5). Thus, as a prerequisite to evaluate binding of L-Fuc, L-Gal, and their methyl pyranosides to PRM-A, we modified the procedure of the co-precipitation experiment.

Among various conditions examined, the best result was obtained when 2.0 μ mol of PRM-A was mixed with 20 μ mol of CaCl₂ and 50 μ mol of each sugar in 900 μ L of distilled water at pH 6.0. Under these conditions, the [PRM-A/Ca²⁺] complex effectively formed the aggregates, which bound similar amounts of D-Man, D-Ara, and α -D-Man-OMe (Table 1). The molar ratio of α -D-Man-OMe in the aggregate (α -D-Man-OMe/PRM-A = 1.13) was almost the same as that obtained in the previous co-precipitation experiment performed at pH 4.5 (α -D-Man-OMe /PRM-A = 1.08).¹² On the other hand, D-Glc as well as methyl α -D-glucopyranoside (α -D-Glc-OMe) was

hardly co-precipitated with PRM-A, suggesting that the adduct formation is successfully suppressed at pH 6.0.

Having confirmed that specific binding of both reducing and non-reducing sugars to PRM-A can be evaluated by this modified procedure, we examined binding ability of PRM-A to L-Fuc, L-Gal, and their methyl pyranosides (L-Fuc-OMe, L-Gal-OMe). In accord with the previous report that BMY-28864 failed to capture L-Fuc and L-Gal,¹⁰ only negligible amounts of L-Fuc and L-Gal were detected in the aggregates (Table 1). However, both anomers of L-Fuc-OMe and L-Gal-OMe were found to exhibit significant binding to PRM-A, though the detected amounts of these sugars were smaller than those of α - and β -D-Man-OMe. It is also interesting that PRM-A preferred the β -anomer to the α -anomer in both L-Fuc-OMe and L-Gal-OMe while such discrimination was not observed in D-Man-OMe. These results implicate the anomeric hydroxyl groups of L-Fuc and L-Gal as key elements in their binding to PRM-A. However, it is unlikely that the anomeric hydroxyl groups sterically or electronically inhibit the interaction of L-Fuc and L-Gal with PRM-A because PRMs accommodate D-Ara and D-fructose (Fig. 3) without any hindrance by their anomeric hydroxyl groups.¹⁰

One remaining possibility is that Ca^{2+} ion traps L-Fuc and L-Gal by chelation, thereby blocking association of these sugars with PRM-A. This hypothesis is based on the crystal structures of Ca^{2+} complexes of D-Fuc and D-Gal, in which one of the sugar molecules chelates Ca^{2+} ion with the anomeric (C1) oxygen atom (Fig. 4).^{14,15} Assuming that the C1 methoxy groups of L-Fuc-OMe and L-Gal-OMe destabilize the

Ca²⁺ complexes by steric reasons, our hypothesis provides a possible explanation for why PRM-A can capture these methyl pyranosides but not L-Fuc and L-Gal. Additionally, when considering that all sugar molecules in the complexes exist as the α -pyranose forms, it is possible to speculate that complex formation of Ca²⁺ ion with the β -anomers is relatively unfavorable, which is consistent with the observation that PRM-A preferably binds the β anomers of L-Fuc-OMe and L-Gal-OMe.

As a test of this hypothesis, we performed co-precipitation experiments using an increased amount of CaCl₂ (100 μ mol) to enhance complex formation of Ca²⁺ ion with L-Fuc-OMe and L-Gal-OMe (Table 1). As expected from our hypothesis, PRM-A was found to lose the ability to capture both anomers of these sugars. On the other hand, incorporated amounts of D-Man and D-Man-OMe were nearly distinguishable between the experiments with 20 and 100 μ mol of CaCl₂, confirming that extra addition of Ca²⁺ ion gives marginal impact on binding of PRM-A with these sugars. Although we were not able to perform experiments using a reduced amount of CaCl₂ (< 10 μ mol) due to poor aggregate formation, these results clearly indicate that Ca²⁺ ion inhibits association of PRM-A with L-Fuc-OMe and L-Gal-OMe.

Based on these results, we next examined whether L-Fuc and L-Gal interacts with Ca²⁺ ion in aqueous solution. It has been shown that complex formation of sugars with metal ions can be detected by changes in equilibrium composition of α - and β -pyranose forms upon addition of metal ions.^{16,17} Earlier studies by ¹H-NMR spectroscopy revealed that Ca²⁺ ion interacts with D-allose but not with D-Glc, D-Man, and D-Ara.¹⁸ We therefore recorded ¹H-NMR spectra of L-Fuc and L-Gal in D₂O containing 0, 0.4,

and 2 equivalents of CaCl_2 (Fig. 5). Control experiments using D-Man demonstrated that the α/β ratio of D-Man was almost maintained upon addition of CaCl_2 , confirming its negligible binding with Ca^{2+} ion under our conditions. On the other hand, α/β ratios of L-Fuc and L-Gal were dependent on Ca^{2+} concentration. With addition of Ca^{2+} ion, proportion of their α forms was significantly increased, and that of the β forms was decreased accordingly. Although no substantial change in chemical shifts of sugars was detected upon addition of Ca^{2+} ion, the realistic implication of these observations is that L-Fuc and L-Gal interact with Ca^{2+} ion in aqueous solution.¹⁹ The shift of the equilibrium toward the α -pyranose forms possibly reflects that the α -anomers of L-Fuc and L-Gal preferentially form the Ca^{2+} complexes as observed in the crystal structures (Fig. 4).

These results collectively support our hypothesis that poor binding of L-Fuc and L-Gal to PRM-A is attributed to their chelation with Ca^{2+} ion. Unlike D-Man and D-Ara, which forms the $[\text{PRM-A}/\text{Ca}^{2+}/\text{Sugar}]$ complex without interruption by Ca^{2+} chelation, L-Fuc and L-Gal possibly form the $[\text{Sugar}/\text{Ca}^{2+}]$ complex rather than the $[\text{PRM-A}/\text{Ca}^{2+}/\text{Sugar}]$ complex (Scheme 1). In the case of L-Fuc-OMe and L-Gal-OMe, destabilization of the $[\text{Sugar}/\text{Ca}^{2+}]$ complex by their C1 methoxy groups enables a part of these sugars to form the $[\text{PRM-A}/\text{Ca}^{2+}/\text{Sugar}]$ complexes, resulting in small but significant incorporation of these sugars into the aggregates. This suggests that PRM-A binds pyranosides of L-Fuc and L-Gal when Ca^{2+} concentration is not excessive to trap these sugars by chelation but sufficient to form the $[\text{PRM-A}/\text{Ca}^{2+}]$ complex. Given that L-fucopyranoside is commonly found as a component of

biologically and pathologically important glycans,²⁰ this study contributes to the better understanding of the mode of biological action of PRMs. Further efforts to examine the binding ability of PRMs for L-Fuc-containing glycans are currently underway.

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Figure and Scheme Captions

Figure 1. Structures of BMY-28864 and pradimicin A (PRM-A).

Figure 2. Binding model of PRM-A with α -D-Man-OMe.¹³

Figure 3. Structural comparison of D-mannose (D-Man) with D-arabinose (D-Ara), D-fructose, D-lyxose, L-fucose (L-Fuc), and L-galactose (L-Gal).

Figure 4. Schematic representation of crystal structures of [D-Fuc/Ca²⁺] (left)¹⁴ and [D-Gal/Ca²⁺] (right)¹⁵ complexes.

Figure 5. ¹H-NMR spectra of D-Man (A), L-Fuc (B), and L-Gal (C) in D₂O containing 0, (bottom) 0.4 (middle), and 2 equivalents (top) of CaCl₂. Anomeric proton regions (4.4 – 5.4 ppm) are shown for ease of comparison (for full spectra, see Supplementary data).

Scheme 1. Possible complex formation of sugars with PRM-A and Ca²⁺ ion.

BMY-28864 : R = OH, X = Me
 Pradimicin A (PRM-A) : R = X = H

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Scheme 1

Table 1. Molar ratio of sugars relative to PRM-A in aggregates.

Sugar	CaCl ₂	
	20 μ mol (0.4 eq. relative to sugar)	100 μ mol (2 eq. relative to sugar)
D-Man	1.07 ^a (0.06) ^b	1.08 (0.03)
α -D-Man-OMe	1.13 (0.02)	1.11 (0.08)
β -D-Man-OMe	1.15 (0.07)	1.08 (0.06)
D-Ara	1.14 (0.03)	NT ^c
D-Glc	< 0.1	NT
α -D-Glc-OMe	< 0.1	NT
L-Fuc	< 0.1	< 0.1
α -L-Fuc-OMe	0.28 (0.03)	< 0.1
β -L-Fuc-OMe	0.52 (0.05)	< 0.1
L-Gal	< 0.1	< 0.1
α -L-Gal-OMe	0.32 (0.03)	< 0.1
β -L-Gal-OMe	0.60 (0.05)	< 0.1

^aThe ratios were calculated from ¹H-NMR data on the basis of integration values of PRM-A and each sugar after dissociation of the washed aggregate by acid treatment. In the case of D-Man, D-Ara, D-Glc, L-Fuc, and L-Gal, the sum of the integration values of α - and β -anomers was used for the calculation.

^bStandard deviation of at least three separate experiments.

^cNot tested.