Tetrahedron 71 (2015) 2603–2609

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

A mannose-recognizable chemosensor using gold nanoparticles functionalized with pradimicin, a nonpeptidic mannose-binding natural product

Masaru Enomoto [a,*], Yasuhiro Igarashi [b], Masahide Sasaki [a], Hiroki Shimizu [a]

[a] Biomaterial Engineering Research Group, Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Toyohira-ku, Sapporo 062-8517, Japan
[b] Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Kurokawa, Imizu, Toyama 939-0398, Japan

ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 13 March 2015
Accepted 15 March 2015
Available online 19 March 2015

Keywords:
Gold nanoparticles
Pradimicin
Chemosensor
Cluster effect
Molecular recognition

ABSTRACT

By making use of the distance-dependent optical properties of gold nanoparticles as well as the mannose (Man)-specific binding ability of pradimicin (PRM), we have successfully developed a Man-recognizable chemosensor. The dissociation constant of the chemosensor for Man was estimated to be approximately 10 μM, which was ten times lower than that of PRM itself. This result may be attributed to a cluster effect. Therefore, the chemosensor was capable of recognizing Man residues that have not yet been identified as the binding sites for PRM.

1. Introduction

Of the various types of glycans that exist in a living organism, a family of high mannose (Man) type glycans is one of the most intriguing. For instance, some of these glycans are known to act as tags to discriminate between misfolded and currently folded glycoproteins.[1,2] One of the most notable examples is the HIV envelope glycoprotein, gp120, whose surface is highly glycosylated.[3–6] The carbohydrate moieties containing high Man-type glycans are believed to shield highly immunogenic epitopes. It is reported that this mechanism makes the HIV virus invisible to the immune system. Despite the biological and medicinal importance of high Man-type glycans, there are only a few known methods that can be used to detect Man residues. One of these methods is the utilization of Man-specific lectins,[7–10] which are commonly used as conventional analytical tools in glycobiology. However, their limited supplies, high manufacturing costs and low chemical stabilities make them an unfavorable choice for use in a feasible Man detection method.[1]

Pradimicins (PRMs), which were isolated from the culture broth of *Actinomyces* species, are a family of natural products with the ability to bind Man residues in the presence of Ca^{2+} ions (Fig. 1).

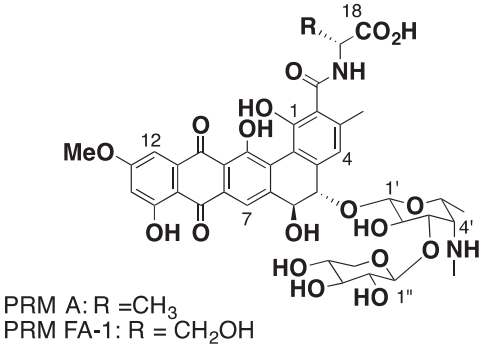
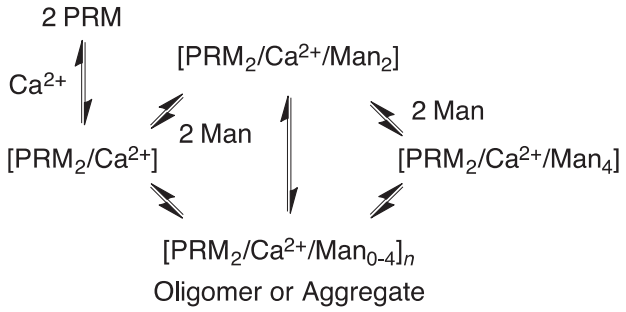


Fig. 1. Structures of PRM A and PRM FA-1.

PRMs exhibit potent antifungal and *anti*-HIV activities by binding to the Man-rich glycans present on fungi or virus surfaces.[11–19] In other words, PRMs can act as lectin-mimics even though they are secondary metabolites. Since PRMs are regarded as promising therapeutic alternatives to Man-specific lectins, many natural product and carbohydrate chemists have tried to elucidate the mechanism of Man recognition.[13,15–23] Nakagawa and co-workers have demonstrated a mechanism involving the interaction of four complexes: $[PRM_2/Ca^{2+}]$, $[PRM_2/Ca^{2+}/Man_2]$, $[PRM_2/Ca^{2+}/Man_4]$ and $[PRM_2/Ca^{2+}/Man_{0\text{-}4}]_n$ (Scheme 1).[21] The $[PRM_2/Ca^{2+}]$ complex initially binds two molecules of Man to form the $[PRM_2/Ca^{2+}/Man_2]$

* Corresponding author. Tel.: +81 11 857 8973; fax: +81 11 857 8980; e-mail address: masaru-enomoto@aist.go.jp (M. Enomoto).

http://dx.doi.org/10.1016/j.tet.2015.03.055
0040-4020/



Scheme 1. Complex and aggregate forming reaction of PRM, Ca^{2+} and Man proposed by Nakagawa and co-workers.

complex, which incorporates another two molecules of Man to create the [$PRM_2/Ca^{2+}/Man_4$] complex. As a consequence of these complex-forming reaction, aggregates consisting of the [$PRM_2/Ca^{2+}/Man_{0\text{-}4}]_n$ complex appear. This complex-forming property as well as the Man-specific recognition ability of PRMs make them ideal candidates used in chemosensors for detecting Man residues.

As a result of their unique properties, detection strategies using gold nanoparticles (AuNPs) have recently attracted a great deal of attention.[24–26] For example, their extinction coefficients are several orders of magnitude higher than those of organic dyes.[27–29] Additionally, the color change of the colloidal solution is known to be dependent on the distance between each AuNP.[27–29] The red color of dispersed AuNPs turns to dark blue upon aggregation. By functionalizing AuNPs with specific host compounds, many chemosensors that can detect gest compounds at low concentrations have been developed.[24–26,28–34] In light of the distance-dependent optical properties of AuNPs combined with the Man-specific binding ability of PRM, PRM-functionalized AuNPs (PRM–AuNPs) are expected to serve as promising chemosensors for Man detection. Our strategy for Man detection is shown in Fig. 2. PRM bound to the surface of AuNPs should cause aggregation in the presence of Man and Ca^{2+} ions. As the nanoparticles bind to one another, an attenuation and red shift in UV–visible absorption spectra should be observed. Oki and co-workers have shown that sugar bindings between PRMs and sugars can be observed by UV–visible spectrophotometric analysis without assistance of AuNPs. We expected that extinction coefficients of AuNPs would enable to detect Man more sensitively. On the other hand, they also reported that since two molecules of PRMs bind Ca^{2+} ion to form [PRM_2/Ca^{2+}],[19] the aggregation can be induced by Ca^{2+} ion only. However, Bewley and co-workers reported that they could not observed large particles in the absence of sugar,[23] which meant that changes of the spectra in the absence of Man could be small because the degree of change of the particle size is reflected in that of the spectra. Thus we speculated that the attenuation caused by Ca^{2+} ion should be negligible for Man detection.

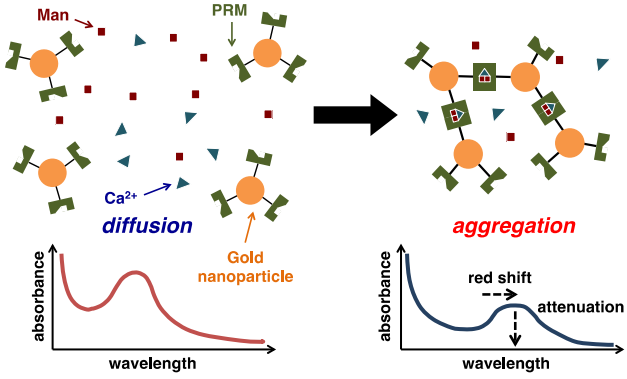


Fig. 2. Overview of the sensing mechanism by the PRM–AuNPs: aggregation of PRMs bound to the surface of the AuNPs in the presence of mannose and Ca^{2+} may cause their proximity to each other and result attenuation and red shift in the UV–visible absorption spectra.

Although PRM may be applied to a useful tool to detect Man residues, it is not fully understood, which Man residues PRM can recognize in glycans. Nakagawa and co-workers have shed some light on the mechanism of the Man recognition by making use of solid-state NMR spectroscopy and isothermal titration calorimetry (ITC).[21] Their extensive study led them to propose a new binding model, which shows that PRM can bind the terminal Man residues at the non-reducing end of glycan as well as internal 6-substituted glycans. On the other hand, Oki's group has reported that the PRM binds to the terminal sites in glycan and that C-6 phosphorylated Man was unrecognizable under UV–visible spectrophotometric analysis.[17,19] Thus only the terminal and internal 6-substituted Man residues have been identified as PRM binding sites so far, which limits the scope in which PRM–AuNPs are applicable. We expect that this limitation can be overcome by the cluster effect,[35–37] which entropically enhances binding and steric stabilization by multivalent interactions and plays important roles in biological process. Since it has been reported that AuNPs possessing numbers of bioactive compounds on their surfaces are subjected to the cluster effect,[37,38] the affinity for Man can be enhanced by conjugating PRM to AuNPs.

Herein we will report on the synthesis of PRM–AuNPs and, subsequently, their scope and limitations as a potential chemosensor for Man detection. To the best of our knowledge, this study is the first example of a secondary metabolite employed as a molecular recognition ligand.[26,39]

2. Results and discussion

2.1. Synthesis of the PRM–AuNPs conjugates

The synthetic procedure for the PRM–AuNP conjugates is shown in Scheme 2. Based on Oki's reports that the most 4′-NHMe modified PRM derivatives exhibited acceptable antifungal activity,[40] we designed **1** as the target compound. Our synthesis of **1** began with the preparation of a linker with an alkynyl group; the purpose of this linker was to connect AuNPs with a PRM moiety via the Click reaction. PRM tends to aggregate at high concentration conditions, which posed a problem with regard to synthesis and storage of the target molecule. To circumvent this issue, we chose to etherify part of the hydroxyl groups included in the linkers, thereby the PRM–AuNPs more hydrophilic and stable. In accordance with this speculation, we carried out mono-mesylation of a known disulfide **2**[41] with the equivalent amount of methanesulfonyl chloride followed by etherification with an excess of propargyl alcohol and sodium hydride. This process afforded **3** in 91% yield, the structure of which was confirmed by NMR and MS analyses. Reduction of $KAuCl_4$ with aqueous $NaBH_4$ in a mixed solvent MeOH/water (15/1) in the presence of **3** proceeded uneventfully to yield **4**, whose diameter was determined to be ca. 15 nm by dynamic light scattering (DLS).[42] We then prepared a PRM derivative bearing an azido group. Condensation of known amine **5**[43] with bromoacetyl bromide gave **6** at a modest yield of 35%. Among a family of PRMs, we chose PRM FA-1 as the host compound in this study because it was more hydrophilic congener among the PRM family due to its C17-hydroxymethylene group, which would result to prevent aggregation of AuNPs. Thus, according to the Oki's procedure for preparing *N*-carboxymethylated PRM derivatives,[40] a mixture of PRM FA-1/A (ca. 3:1)[44] in CH_2Cl_2 was treated with *N*, *O*-bis(trimethylsilyl)acetamide and **6** in a screw-capped vial at 60 °C for 3 days to give the desired **7** at 9% yield along with 3% of an *N*-carboxymethylated PRM A derivative corresponding to **7** and 27% of recovered PRM FA-1/A. This low yield compared to that reported by Oki's group might be due to the low reactivity of **6**: they used α-iodoacetamide for the alkylation, which is simpler and more