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Complexation of antimony (Sb^V) with guanosine 5′-monophosphate and guanosine 5′-diphospho-D-mannose: Formation of both mono- and bis-adducts

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Abstract

In spite of the extensive use of pentavalent antimony chemotherapy, the mechanism of its anti-leishmania action is still not clear. Here, we report the interactions of Sb^V, including the clinically used drug stibogluconate, with guanosine 5′-monophosphate (5′-GMP) and guanosine 5′-diphospho-D-mannose (5′-GDP-mannose) in aqueous solution. The deprotonated hydroxyl groups (–OH) of the ribose ring are shown to be the binding site for Sb^V, probably via chelation. Both mono- and bis-adducts were formed as determined by NMR, high performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS), and both of them are stable in the pH range of 4 to around 9.5. The formation of the mono-adduct ($k_1 = 1.67 \times 10^{-3}$ and 3.43×10^{-3} mM^{-1} min^{-1} for Sb(5′-GMP) and Sb(5′-GDP-mannose), respectively, at 298 K) was 10-fold faster than that of the bis-adduct ($k_2 = 0.16 \times 10^{-3}$ and 0.21×10^{-3} mM^{-1} min^{-1}, for $Sb(5'\text{-GMP})_2$ and $Sb(5'\text{-GDP-mannose})_2$, respectively), and the mono-adduct was the major species in solution with the [bis-adduct]/[mono-adduct] < 0.5. The reactions of stibogluconate with 5′-GMP and 5′-GDP-mannose were slower than that of antimonate under similar conditions.

Keywords: Antimony; ESI-MS; Guanosine 5′-monophosphate; Guanosine 5′-diphospho-D-mannose; *Leishamania*; NMR

1. Introduction

Leishmaniasis has been attested in 88 countries. Approximately 1.5 billion individuals are at risk from this disease and 400,000 cases are reported annually [1,2]. Pentavalent antimony (Sb^V) compounds such as sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®) are the first choice of therapy for leishmaniasis [3,4]. In spite of their extensive clinical use for several decades, the mechanism of action is still not clear [5].

In contrast to mammalian cells, all protozoan parasites studied to date are unable to synthesize the purine ring de novo. Instead, they have evolved as a unique purine salvage strategy which is critical for their survival. The first step of the salvage process of *Leishmania donovani* is the translocation of preformed purines [6]. The uptake of trivalent arsenical drug (an analog of antimony) by *Trypanosoma brucei* was regulated by a P2 nucleoside transporter [7] and other anti-leishmanial agents such as purine analogs allopurinol riboside were also manipulated by these specific transporters [8]. Pentavalent antimony may form a complex with adenine in vivo within the acidic cell compartments [9]. Selective interference with this novel salvage pathway, which is biochemically different between the parasite and the host, may be one of the actions of antimony drugs.

The mannose-containing glycoconjugate pathway in *Leishmania* species has attracted a considerable attention in searching for the mechanisms of action of antimony drugs. *Leishmania* species synthesizes a number of unusual

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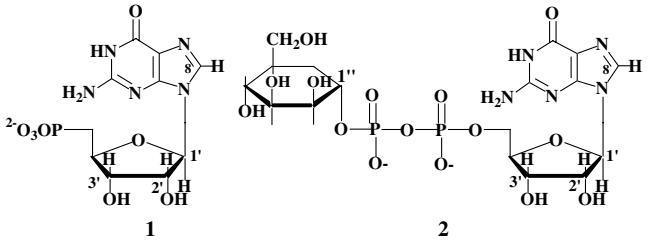
mannose-rich glycoconjugates on the cell surface, which possess a variety of important functions [10,11]. Since the activated mannose donor for all mannosylation reactions in *Leishmania* is 5′-GDP-mannose, the synthesis of all important mannose containing biomolecules directly or indirectly depends on the availability of 5′-GDP-mannose. 5′-GDP-mannose is synthesized at cytoplasm, so it is required to transport the nucleotide-sugar to the Golgi apparatus via a golgi 5′-GDP-mannose transporter known as LPG2 [12,13]. This transporter is only expressed in certain lineages, for example, in yeast and *Leishmania*, but is not expressed in mammalian cells [14]. It has also been shown that although 5′-GDP-mannose nucleotide-sugar transporter activity was not required for the growth of *Leishmania* in vitro, it is essential for the synthesis of mannose-rich molecules, and this is important for the virulence of the parasites [15]. This offers an attractive target for the anti-leishmania therapeutics of antimony agents.



This paper reports the investigation of the complexation of antimonate (Sb^V) to 5′-GMP (**1**) and 5′-GDP-mannose (**2**), and characterization of Sb^V adducts by ESI-MS spectrometry, HPLC and NMR spectroscopy. Both mono- and bis-adducts were observed with the former being the major component.

2. Results

2.1. Sb^V binding to 5′-GMP and 5′-GDP-mannose by NMR spectroscopy

^{1}H NMR was used to identify the antimony nucleotide derivative interaction, and the ^{1}H NMR spectra of 5′-GMP (**1**) and 5′-GDP-mannose (**2**) were assigned according to previous reports (Table 1) [16]. Addition of 2.5 mM antimonate (Sb^V) into **1** or **2** solution (a molar ratio of 1:2) at pH 5.0 after equilibration for 20 h resulted in two sets of new resonances for H'_1 and H'_2 (Fig. 1), indicative of formation of mono- and bis-adducts of Sb^V and **1** or **2** (vide infra).

In order to assign these proton resonances, particularly in the ribose ring, various 2D NMR experiments were employed. A 2D [^{1}H, ^{13}C] total correlation spectroscopy-heteronuclear single quantum correlation (TOCSY-HSQC) spectrum of the Sb^V and **1** mixture (10 mM at a molar ratio of 1:1) at pH 5.0 and 310 K showed correlations between H'_1 and H'_4 and even H'_5 protons of the ribose ring corresponding to a specific carbon, e.g., C1′ of the ribose ring (90.7 ppm) (Fig. 2). New resonances for H'_1 at 5.67 and 5.87 ppm exhibited cross-peaks with several carbons of the ribose ring in addition to their directly attached carbon (C1) at 87.7 and 90.6 ppm. The observed three sets of both ^{1}H and ^{13}C peaks confirmed the presence of three major species in solution, presumbly one free and two bound species. These peaks can therefore be assigned, and the detailed assignment is summarized in Table 1. A 2D [^{1}H, ^{13}C] TOCSY-HSQC experiment of the Sb^V and **2** mixture was also carried out under identical conditions and the assignment is also summarized in Table 1. Evident changes in shifts were observed for H'_1 and H'_2 for both **1** (coordinated shift $\Delta\delta = -0.27$ and 0.32 ppm for H'_1 and H'_2, respectively) and **2** ($\Delta\delta = -0.27$ and 0.33 ppm). By contrast, only minor changes for H_8 ($\Delta\delta = 0.060$ and 0.080 ppm for **1** and **2**, respectively) were noted. In addition, the changes in shifts for H'_1 and H'_2 in one species were much larger than the other. The large changes for H'_2 suggest that −OH group(s) in the ribose are the binding sites for Sb^V probably via ring chelation (at C2′ and C3′). This

Table 1
^{1}H NMR chemical shifts for the free 5′-GMP as well as its mono- and bis-adducts[a]

Protons	Free 5′-GMP	Sb^V (5′-GMP)	Sb^V (5′-GMP)$_2$	$\Delta\delta_1$	$\Delta\delta_2$
H'_1	5.94	5.87	5.67	−0.07	−0.27
H'_2	4.73	4.76	5.05	0.03	0.32
H'_3	4.47	4.46	4.41	−0.01	−0.06
H'_4	4.33	4.27	4.29	−0.06	−0.04
H'_5	4.11	4.14, 4.16	4.04, 3.96	0.03, 0.05	−0.07, −0.15
H_8	8.10	8.11	8.16	0.01	0.06

[a] $\Delta\delta = \delta_{(\text{adduct})} - \delta_{(\text{free ligand})}$.

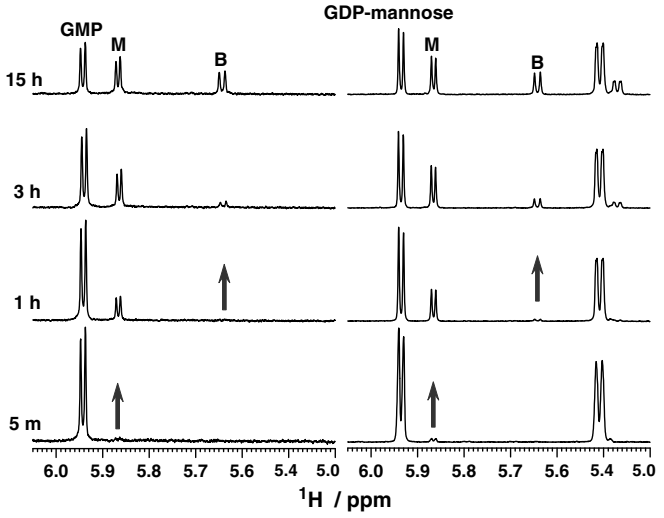


Fig. 1. Partial 600 MHz ^{1}H NMR spectra of Sb^V–5′-GMP mixture (2.5:5 mM) (left) and Sb^V–5′-GDP-mannose mixture (2.5:5 mM) (right) at different time at pH 5.0 and 298 K. "M" and "B" represent the concentration of mono- and bis-adducts (Sb^V (5′-GMP)$_n$ or Sb^V (5′-GDP-mannose)$_n$, $n = 1, 2$), respectively.