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Synthesis and characterization of Sb(V)–adenosine and Sb(V)–guanosine complexes in aqueous solution

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Abstract

Sb(V) is known to form a complex with adenine ribonucleosides suggesting that ribonucleosides may be involved in the mechanism of action of pentavalent antimonial drugs against the parasitic disease leishmaniasis. In this study, Sb(V) complexes with adenosine and guanosine were prepared and characterized. Two Sb(V)–adenosine complexes were obtained in the solid state with either 1:2 or 1:1 Sb(V):adenosine molar ratios. A thermoreversible Sb(V)–guanosine hydrogel was also obtained using Sb:guanosine molar ratios varying from 0.5 to 1. These complexes were characterized by H^1 NMR spectroscopy, high resolution eletrospray ionization mass spectrometry, elemental analysis and circular dichroism. For the adenosine complexes, we propose that Sb(V) is either penta-coordinated by two riboses and one hydroxyl anion or octa-coordinated by two riboses and two hydroxyls or by one ribose and four hydroxyls. The Sb(V)–guanosine hydrogel is shown to be composed of a mixture of 1:1 and 1:2 Sb(V)–guanosine complexes, forming nanoassemblies with two types of interactions: (i) covalent bonds forming Sb(V)–guanosine complexes and (ii) intermolecular interactions between the different Sb(V)–guanosine complexes via base stacking.

Keywords: Nucleoside; Antimony; Circular dichroism; ESI-MS; NMR; Leishmaniasis

1. Introduction

Despite their clinical use for more than half a century, the mode of action of pentavalent antimonials against leishmaniasis remains poorly understood [1,2]. A major question still to be answered is whether the final active form of pentavalent antimonials is composed of Sb(V) or Sb(III). It has been reported that part of Sb(V) is reduced in vivo into more toxic Sb(III) [2–4]. Recent studies also suggested that thiols act as a reducing agent for Sb(V) to Sb(III) conversion [5–7]. Recently, however, a Sb(V) complex with adenine ribonucleoside in aqueous solution has been reported [2,8], showing for the first time a physiologically relevant biomolecule capable of forming stable Sb(V) complexes. Circular dichroism data were consistent with the formation of 1:2 Sb–ribonucleoside complexes, whereas NMR data indicated that Sb(V) is bound to the sugar portion at the 2′ position, but full structural characterization of these Sb(V)–ribonucleoside complexes has not been yet performed. Considering that guanosine and derivatives readily form supramolecular aggregates in aqueous solutions [9], the effects of complexation of guanosine with Sb(V) should also be investigated.

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Herein, we report the preparation of 1:2 and 1:1 Sb–adenosine complexes and their detailed structural characterization by NMR and ESI-MS techniques. We also show that Sb(V) forms complexes with guanosine in aqueous solution resulting in a thermoreversible hydrogel in which two types of interaction occur: covalent bonds forming Sb(V)–guanosine complexes and intermolecular interactions between the different Sb(V)–guanosine complexes via base stacking.

2. Experimental

2.1. Materials

Adenosine (Ad), guanosine (G) and guanosine 5′-monophosphate (GMP) were obtained from Sigma Chemical Co. (St. Louis, MO). Potassium hexahydroxoantimonate ($KSb(OH)_6$) was obtained from Fluka Chemie GmbH (Switzerland). All other reagents were of at least reagent grade. Double-distilled-deionized water was used throughout all the experiments.

2.2. Preparation of the Sb(V)–adenosine complexes

2.2.1. From acidic medium

About 4.5 mmol of Ad was dissolved in 150 mL of water and 2.3 mmol of $KSb(OH)_6$ was added to this solution. The pH of the mixture was adjusted to 5.0 and the solution was kept under stirring at 25 °C. After 24 h, a white precipitate was formed. This precipitate was washed with acetone and dried over $CaCl_2$, and the product was recovered which gives a reaction yield of 11%.

Anal. Calc. for $C_{20}H_{33}N_{10}O_{14}Sb$ ($C_{20}H_{23}N_{10}O_9Sb + 5\text{-}H_2O$), m.p. ~225 °C (dec): C, 31.63; H, 4.34; N, 18.45; Sb, 16.04. Found: C, 31.36; H, 4.27; N, 17.99; Sb, 17.03%.

Note. Following the above procedure but using instead 4.5 mmol of Ad and 4.5 mmol of $KSb(OH)_6$ (1:1 molar ratio) and precipitation with acetone led to the formation of poorly water-soluble species, presumably with a polymeric structure.

2.2.2. From neutral medium

About 4.5 mmol of Ad was dissolved in 150 mL of water and 2.3 mmol of $KSb(OH)_6$ was added to this solution. The pH of the mixture was adjusted to 7.0 and the solution was kept under stirring at 25 °C. After 1 day, a white precipitate was obtained from precipitation with acetone. This was dried over $CaCl_2$, giving a reaction yield of 21.8%. *Anal.* Calc. for $C_{10}H_{17.4}N_5O_{9.2}SbK$ ($C_{10}H_{15}N_5O_8SbK + 1.2H_2O$), m.p. ~225 °C (dec): C, 23.28; H, 3.37; N, 13.58; Sb, 23.63; K, 7.59. Found: C, 22.93; H, 3.30; N, 13.50; Sb, 24.3; K, 7.72%.

2.3. Preparation of Sb(V)–guanosine hydrogel

G and $KSb(OH)_6$ were co-dissolved in H_2O, 90/10% of H_2O/D_2O or 99.99% of D_2O at 60 °C and pH was adjusted to 5. The resulting mixture was kept under stirring at 60 °C for 1.5 h. After cooling at 25 °C a translucent hydrogel was obtained. In nuclear magnetic resonance (NMR) experiment, three different solutions with nucleoside concentration of 1, 15 and 30 mmol/L were prepared. For electrospray ionization mass spectrometry (ESI-MS) analysis, samples prepared in H_2O were freeze-dried and then reconstituted in H_2O/CH_3OH (1:1, v/v) solution. For circular dichroism (CD) study, the Sb/G gel was diluted in H_2O at a final nucleoside concentration of 1 mmol/L. For photon correlation spectroscopy (PCS) analysis, samples were diluted in water at nucleoside concentration of 8 mmol/L and then submitted to ultrasonication (vibracell ultrasonic processor) for 1 min at 4 °C.

2.4. General experimental techniques

C, H and N analyses were carried out using a Perkin–Elmer 240 Elemental Analyzer. Antimony content was determined by atomic absorption using a HITACHI Z 8200 spectrophotometer. Conductivity data were obtained using a Digimed DM31 conductivimeter. TG was obtained using a Shimadzu TGA-50 instrument under a nitrogen atmosphere.

^1H NMR spectra for adenosine and its Sb(V)-complexes were obtained on a Brüker DRX400-*AVANCE* spectrometer operating at 400.129 MHz using D_2O as solvent. TMS(3-(trimethylsilyl)propionic-2,2,3,3-$d_4$acid, sodium salt) was used as an internal reference.

^1H NMR spectra for G and its hydrogel with Sb(V) were recorded on a 11.7 T (500 MHz) Varian Unity INOVA spectrometer operating with a 5-mm gradient indirect detection probe at different temperatures ranging from 10 to 60 °C. Chemical shifts were referenced to internal signal of water (4.78 ppm at 25 °C, calibrated with an ethylene glycol test tube).

^1H NMR (500 MHz) spectra in D_2O (32 transients) were obtained with a 8-μs pulse length at 58 dB, 1 s of relaxation delay, 4000 Hz spectral width and 16 K data size. The residual water signal was suppressed by a presaturation pulse of 1.5 s during the relaxation delay. The free induction decay (FID) was processed with an exponential multiplication corresponding to 0.3 Hz line broadening prior to Fourier transform. COSY spectra were performed with 128 t1 increments of 16 transients. All FIDs were acquired using 2K data points and data were processed with a shifted sine bell function in both dimensions. A TOCSY experiment was recorded using a 250-ms MLEV-17 spin lock [10]. Data were acquired with 2048 points and 128 tl increments of 16 transients each. In the experiments performed in H_2O/D_2O (90%/10%), water suppression was achieved with either the jump and return sequence [11] or the watergate sequence [12].

ESI-MS analysis were performed on a 2000 QTrap Applied Biosystem mass spectrometer, and detailed operation conditions are described elsewhere [13]. The tandem mass

spectrometric (MS/MS) experiments were performed using ESI and Q1 mass selection of the desirable product ion, q2 collision-induced dissociation (CID) with N_2, and linear ion-trap mass analysis of the CID ionic fragments. The collision energy ranged from 10 to 25 eV, depending on the dissociation liability of the precursor ion. ESI(−)-MS were acquired using H_2O/CH_3OH (1:1, v/v) solutions of each compound and spraying the solution mixture through the ESI source at 25 °C. Values of *m*/*z* are reported for the principal ion, that is, for the most abundant isotopologue of the isotope cluster.

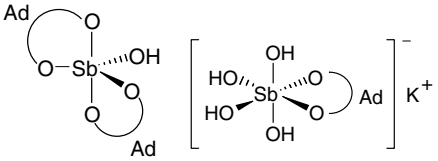
CD spectra were recorded at 25 °C on a Jobin Yvon-Spex Mark CD6 dichrograph, using cuvette path length of 0.1 cm (for 1 mmol/L nucleoside concentration). CD signal is given as $\Delta\varepsilon$, which is the differential molar dichroic absorption coefficient ($\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ in L cm^{-1} mol^{-1}) and is expressed in terms of the molar concentration of the nucleoside.

Photon correlation spectroscopy (PCS) analysis were performed at a 90° scattering angle using a channel correlator (Malvern Instruments type Zetasizer 3000HS) in conjunction with a He/Ne laser (wavelength 633 nm, nominal power output 32 mW).

3. Results and discussion

3.1. Characterization of Sb(V)–adenosine complexes

From the reaction of $KSb(OH)_6$ with adenosine (Ad) in acidic and neutral aqueous solutions, products of formulae $SbAd_2(OH)$ (**a**) and $KSbAd(OH)_4$ (**b**), were obtained.



Conductivity measurement of 1 mmol/L water solution of **b** gave 90 μs/cm, which indicated the presence of ionic species. On the other hand, conductivity measurements performed for **a** in water confirmed that it is constituted essentially of non-ionic species.

ESI-MS were attempted for structural characterization of compounds **a** and **b**. This technique is characterized by the gentleness by which gaseous ions are formed, by ion evaporation directly from solution [14], by allowing handling of loosely bonded ionic species [15,16], by reflecting closely the ionic composition of the solution, and by detecting neutrals as their ionized forms arising from acid–base equilibria [17]. This technique has been used with great success for the detailed structural analysis of metallo-organic species of many classes [18–23]. The ESI mass spectrum in the positive ion mode showed no detectable antimony ions for solutions of **a** and **b** but, when the negative ion mode was used, important anionic antimony species were clearly detected. Fig. 1(a) shows the ESI(−) mass spectrum of a water/methanol solution of **a** in which the hydroxy adduct of **a**, that is $[SbAd_2(OH)_2]^-$ of *m*/*z* 685, is detected as the main Sb-containing anion. Note the distribution of isotopologue ions very characteristic for a ^{121}Sb (100%)/^{123}Sb (74.8%)-containing ion. By analogy with the acid-base equilibrium between $Sb(OH)_5$ and $Sb(OH)_6^-$ [24], $[SbAd_2(OH)_2]^-$ can be formed by the following acid–base reaction, and be therefore in equilibrium with neutral **a** in aqueous solution:

$$SbAd_2(OH) + H_2O \rightleftharpoons [SbAd_2(OH)_2]^- + H^+ \quad (1)$$

In the spectrum of Fig. 1(a), other minor antimony anions were also detected: $[SbAd(OH)_4]^-$ of *m*/*z* 454, $[O{=}SbAd(OH)_2]^-$ of *m*/*z* 436, and $[(O{=})_2SbAd]^-$ of *m*/*z* 418. Although in-source CID during the ESI process [25] cannot be discarded completely, the mild ESI conditions used indicate that these 1:1 Sb–adenosine complex anions resulted most likely from the equilibrium reaction between 1:2 and 1:1 complexes in aqueous solution (see discussion for the ESI-MS spectrum of **b** below).

Fig. 1(b) shows the ESI(−) mass spectrum for **b**, in which **b** anion, that is $[SbAd(OH)_4]^-$ of *m*/*z* 454, the mono-dehydrated form of **b**, that is $[O{=}SbAd(OH)_2]^-$ of *m*/*z* 436, and the di-dehydrated form of **b**, that is $[(O{=})_2SbAd]^-$ of *m*/*z* 418, are detected as the major anions. The detection of the hydroxy adduct of **a**, that is $[SbAd_2(OH)_2]^-$ of *m*/*z* 685 as a minor ion, indicates again that an equilibrium reaction between 1:2 and 1:1 complexes occurs in aqueous solution. Each of these anions was then mass-selected and structurally characterized via collision-induced dissociation (CID) with nitrogen in ESI-MS/MS measurements. The ESI-MS/MS experiments for ion of *m*/*z* 685 shows a loss of water to give the fragment ion of *m*/*z* 667 as the main fragment path as well as losses of adenine to give *m*/*z* 550, followed by losses of water (*m*/*z* 532) and another neutral adenine (*m*/*z* 415). Herein, the ion of *m*/*z* 454 shows losses of water to afford the fragment ions of *m*/*z* 436 and 418, and the ion of *m*/*z* 436 only gives *m*/*z* 418 in the CID experiment by loss of water. Finally, the ion of *m*/*z* 418 gives as the sole fragmentation ion the adenine anion of *m*/*z* 134.

The two new complexes were further characterized by ^{1}H NMR. Fig. 2 shows the ^{1}H NMR spectra obtained for **a** and **b** in D_2O, whereas Table 1 summarizes the attribution of their ^{1}H NMR resonances. The spectrum for **b** supports the existence of a single species. That for **a**, however, indicates a mixture, as also indicated by its ESI-MS spectrum (Fig. 1(a)). Indeed, the displacement of acid–base equilibrium (1) and the partial dissociation of 1:2 complex into more soluble 1:1 complex are expected to occur during the dissolution of **a** in water.

The chemical shifts of Ad and **a** are consistent with those determined previously for pH 5 solutions of Ad and its 1:2 Sb(V)–Ad complex in D_2O [8]. The change in ^{1}H NMR resonances related to the ribose moiety can be attributed to the

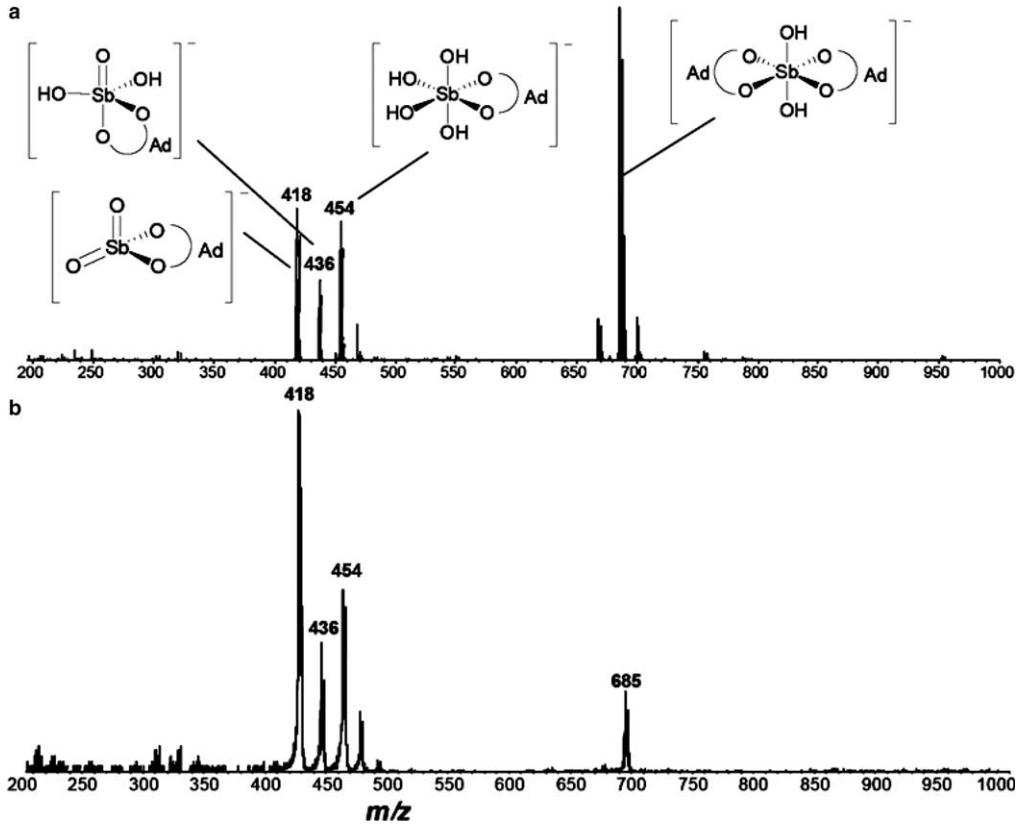


Fig. 1. ESI(−) mass spectra for water/methanol solutions of the Sb–adenosine (Ad) complexes: (a) **a** and (b) **b**. The proton source for the deprotonated species are not indicated.

binding of Sb(V) at 2′ position [8] and, presumably, at 3′ position. However, changes in the ribose conformation also contributed to these alterations, as indicated by the large change in H1′ resonance. Base stacking, as evidenced previously by circular dichroism changes [8], probably contributed to the changes in ribose conformation.

The ^{1}H NMR parameters for **b** are, however, very close to those for Ad, suggesting that the nucleoside conformation remains almost unaffected upon binding of Sb(V). Nevertheless, the downfield shift of 0.12 ppm observed for H2′ resonance (Table 1) indicates that Sb(V) is most probably linked to the 2′ position via an oxygen atom. This observation is important since it is the first experimental evidence that 1:1 Sb(V)–ribonucleoside complex are indeed formed in aqueous solution. Previously [8], we could only identify a 1:2 complex in acidic aqueous medium. The formation of a 1:1 complex in aqueous solution at pH 7 may have important pharmacological implications, especially with respect to the mechanism of action of pentavalent antimonial drugs.

3.2. Characterization of Sb(V)–guanosine hydrogel

The reaction of $KSb(OH)_6$ with guanosine (G) in water, using Sb(V)/G molar ratios varying from 0.5 up to 1, led to the formation of a translucent thermoreversible hydrogel. After freeze-drying and reconstitution with water, the Sb(V)/G mixture recovered as a hydrogel. This behavior is in contrast with that of G alone, which shows significant solubility in water only upon heating and precipitates upon cooling at room temperature.

Fig. 3 shows the ESI(−) mass spectrum for a water/methanol solution of the Sb(V)–G hydrogel. Three major Sb(V)–G complex anions were clearly detected: hydroxy adducts of $[SbG_2(OH)]$ and $[SbG(OH)_2]$, that is $[SbG_2(OH)_2]^-$ and $[SbG(OH)_3]^-$ of m/z 717 of m/z 453, respectively, and the deprotonated form of $[SbG(OH)_2]$, that is $[O{=}SbG(OH)]^-$ of m/z 435.

To investigate further the molecular composition of the hydrogel, 1D and 2D ^{1}H NMR data were collected. The assignment of the chemical shifts of the different protons present in the Sb(V)–G hydrogel was accomplished using 1D proton spectra and 2D COSY and TOCSY correlations. Two samples were used in parallel in these experiments: 1:1 Sb–G hydrogel and G solutions, both prepared in D_2O at the same nucleoside concentration. NMR spectra were also registered in H_2O to get additional information about labile protons in the gel. However, the imino proton region showed only one large peak (data not shown) with insufficient resolution to observe the chemical shifts from the different labile protons.

Fig. 4 displays the 500 MHz ^{1}H NMR spectrum for Sb(V)–G hydrogel at a molar ratio of 0.85 in D_2O. Two particularly interesting regions were seen: the aromatic

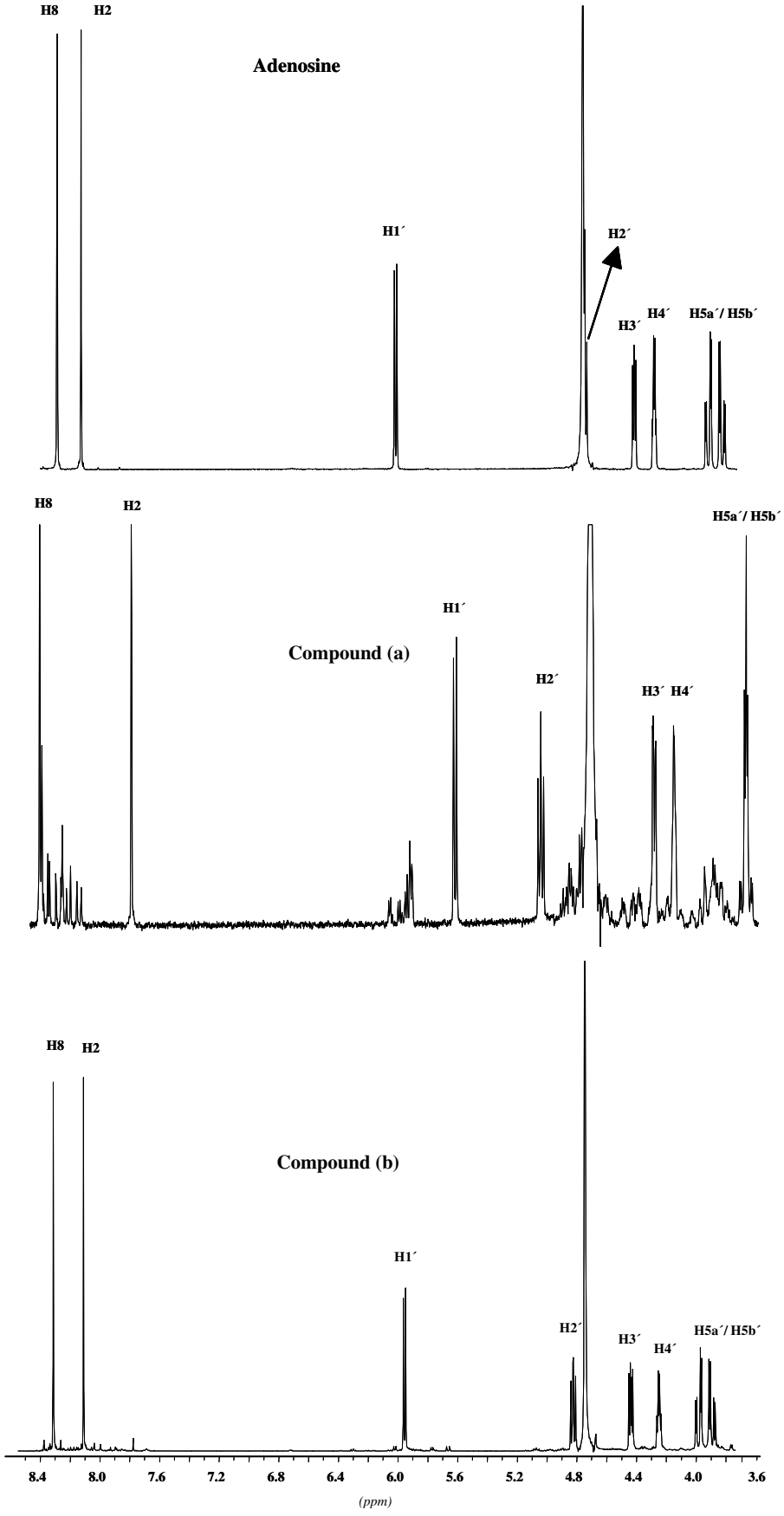


Fig. 2. ^{1}H NMR spectra in D_2O for adenosine, **a** and **b** at pD 6.0.

Table 1
^{1}H NMR parameters (δ, chemical shifts; $^3J_{Hi,Hi+1}$, vicinal coupling constants) obtained at 400 MHz for adenosine (Ad), **a** and **b** in D_2O at pD = 6.0

Proton	Ad		Compound (**a**)		Compound (**b**)		$\Delta\delta$	
	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	A/(**a**)	A/(**b**)
H1′	6.02	5.64	5.64	7.76	5.95	5.6	−0.38	−0.07
H2′	4.85–4.70	–	5.07	6.36, 7.06	4.82	5.6, 6.88	0.37	0.12
H3′	4.44	5.14/3.56	4.33	6.36, 1.84	4.43	6.88, 4.04	−0.11	−0.01
H4′	4.31–4.29	3.22/2.72/3.60	4.15–4.23	1.84	4.26–4.23	4.04, 2.74, 4.33	−0.18	−0.06
H5′	3.94	12.9/2.72	3.74	12.57/4.24	3.98	12.66/2.73	−0.2	0.04
H5′′	3.85	12.80/3.60	3.69	12.57/3.6	3.89	12.66/4.33	−0.16	0.04
H2	8.11	–	7.79	–	8.10	–	−0.32	−0.01
H8	8.27	–	8.39	–	8.31	–	0.11	0.04

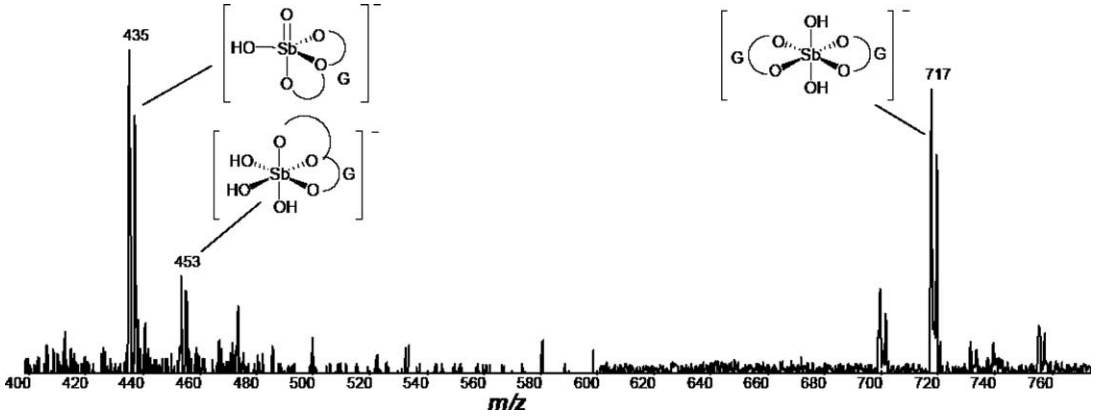


Fig. 3. ESI(−) mass spectrum for a water/methanol solution of the Sb(V)–G hydrogel using a molar ratio of 0.85.

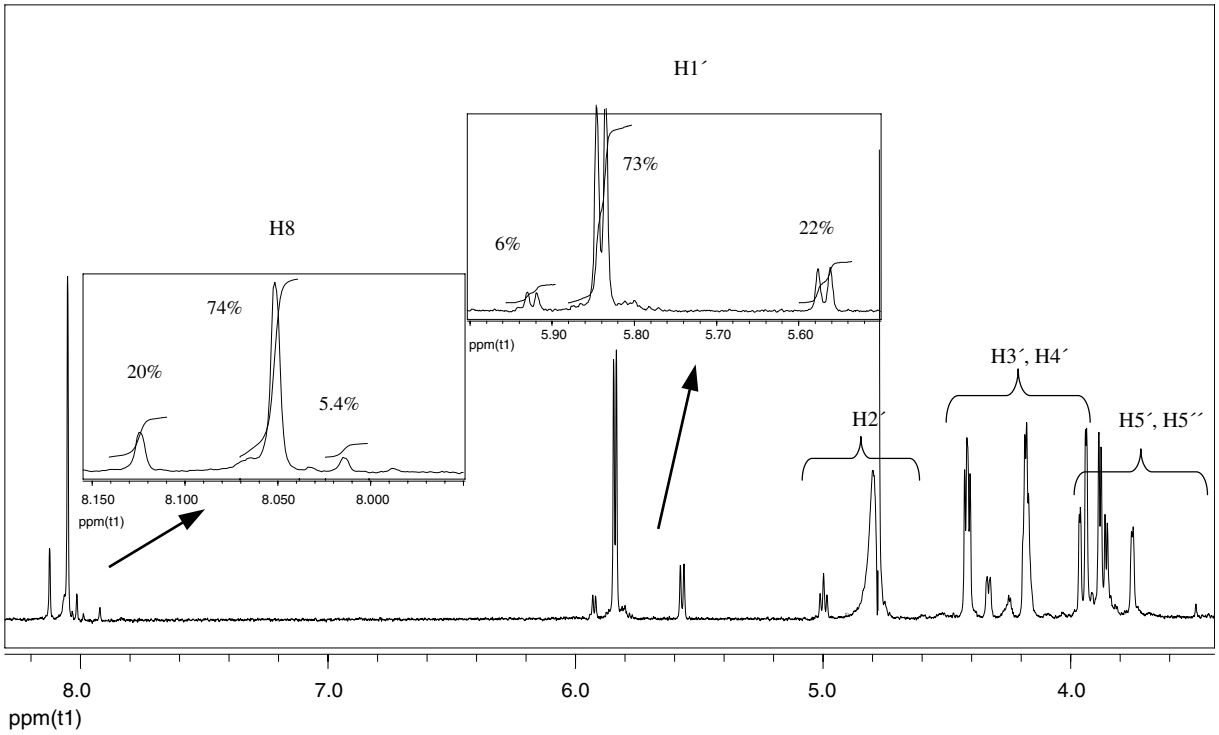


Fig. 4. 500 MHz ^{1}H NMR spectrum obtained for Sb(V)–G hydrogel in D_2O at 25 °C (molar ratio of 0.85; 15 mmol/L of G). Anomeric and aromatic regions with ^{1}H integrations are shown in extensions.

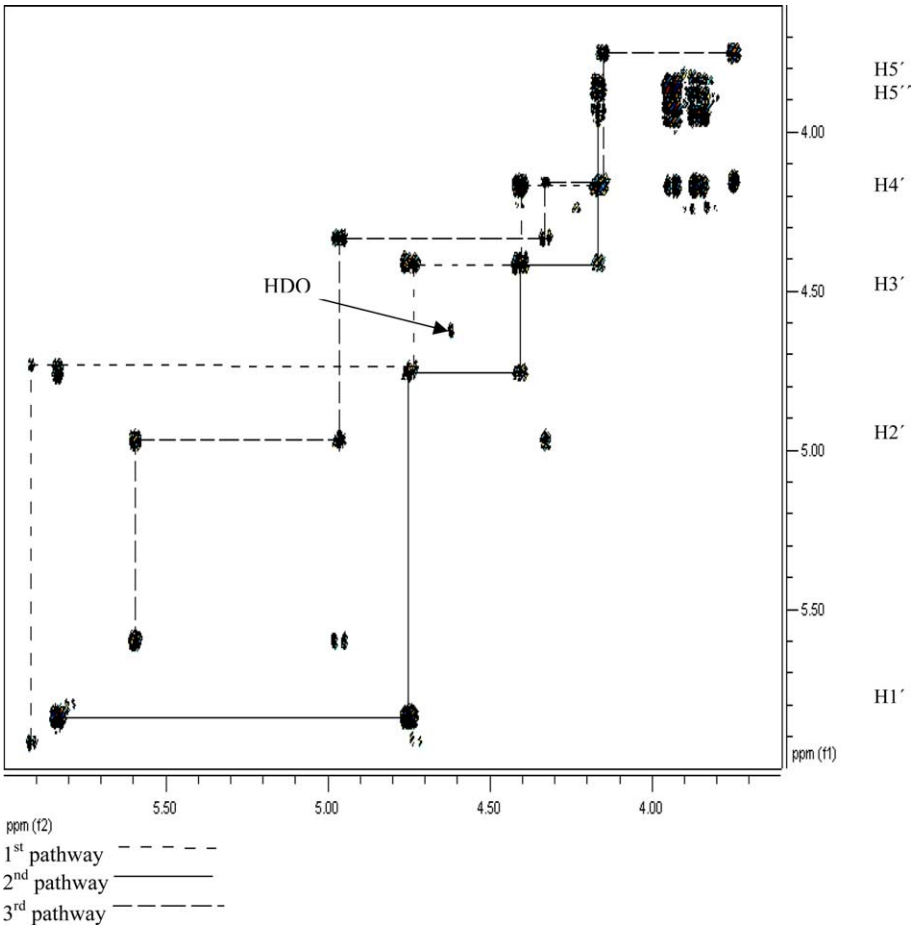


Fig. 5. Ribose region of 500 MHz COSY spectrum obtained for Sb(V)–G hydrogel in D_2O at 40 °C (molar ratio of 0.85; 15 mmol/L of G).

region (7.8–8.2 ppm) and the H1′ region (5.4–6.2 ppm). Each of these regions showed three peaks, including one peak related to "free" G. These three kinds of ^{1}H pathways were also observed in the COSY spectra (Fig. 5). Table 2 summarizes the chemical shifts and vicinal coupling constants determined for G and their antimony complexes.

The two other spin systems, which differed from that of G, most probably correspond to two different Sb(V)–G complexes, **1** and **2**, present in the gel structure. Strikingly, the shifts of ^{1}H NMR signals related to the nucleobase and the ribose are much more pronounced for **2** than for **1** (Table 2). The most important changes concerned H1′ and H2′ resonances, which are most probably related to conformational changes in the nucleoside molecule as well as to the binding of Sb(V) at 2′ position, likewise as demonstrated previously for the Sb(V)–Ad complex [8]. In fact, the three populations mentioned above have different $^3J_{H1'H2'}$ coupling constants (Table 2) supporting the idea that each species has a distinct sugar conformation [26].

From both the NMR and ESI-MS data, the existence of a mixture of 1:2 and 1:1 Sb(V)–G complexes can be proposed. The participation of these antimony complexes in the hydrogel formation is also supported by the observation that the NMR peak pattern is very similar to that of a mixture of 1:2 and 1:1 Sb(V)–Ad complexes.

When we varied the nucleoside concentration from 1 to 30 mmol/L, the peak pattern of the anomeric region remained the same; however, a fourth peak appeared at 30 mmol/L concentration, which we attribute either to a third Sb(V)–G complex or to a fraction of G "trapped" within the hydrogel (data not shown).

From the integration of aromatic and anomeric signals of the spectrum (as shown as an extension in Fig. 4), the proportions of the two complexes were determined. Assuming that the formation of 1:2 Sb(V)–G complex is accompanied by a shift of proton resonances larger than that seen when the 1:1 Sb(V)–G complex is formed, owing to more pronounced changes in the nucleoside conformation [8], the H8 and H1′ peaks at 8.14 and 5.57 ppm can be attributed to the 1:2 Sb(V)–G complex. This species is found to be present at 20% at 25 °C, whereas the major component (74%) is identified as a 1:1 Sb(V)–G complex (H8: 8.06 ppm, H1′: 5.85 ppm). The free form of G was present at near 6%. When the temperature is raised from 10 to 60 °C, an increase of "free" G component and a decrease of the 1:2 Sb(V)–G complex were observed (data not shown).

To investigate the formation of nanoassemblies from the gel, photon correlation spectroscopy was used. When the gel was diluted in water at 8 mmol/L G concentration and

Table 2
500 MHz ^{1}H NMR parameters obtained for Sb(V)–G hydrogel (molar ratio of 0.85) and free G in D_2O, at 15 mmol/L nucleoside concentration (δ, chemical shifts; $^3J_{Hi,Hi+1}$, vicinal coupling constants; $\Delta\delta$, variation of the chemical shift between G and Sb–G species)

Proton	Species in Sb(V)/G hydrogel								G in D_2O		$\Delta\delta$		
	(1)		(2)		(3)		(4)[a]						
	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	δ (ppm)	$^3J_{Hi,Hi+1}$ (Hz)	G/(1)	G/(2)	G/(3)	G/(4)
H8	8.06		8.14		8.03		8.04		8.01		−0.05	−0.13	−0.02	
H1′	5.85	5.53	5.57	7.22	5.94	6.01	5.82		5.93	5.99	+0.08	+0.36	−0.01	−0.11
H2′	4.75		4.99	13.89	4.78		4.87		(4.75)	11.8	0	+0.24	−0.03	−0.12
H3′	4.41	4.43/6.92	4.33	2.48/6.77	4.42		4.27		4.42	3.72/4.96	−0.01	−0.09	0	−0.15
H4′	4.19		4.16		4.24		4.18		4.25	3.60	−0.06	−0.09	−0.01	
H5′/H5′′	3.96/3.88	4.84/12.72, 2.94/12.72	3.75	3.43	3.86		3.93		3.90/3.83	2.89/12.72, 3.80/12.72	−0.06/−0.05	+0.08	−0.03	−0.03
%[b]	74%		20%		6%									
Model[b]	1:1 Sb(V)–G complex		1:2 Sb(V)–G complex		Free G		G ''trapped'' within hydrogel		Free G					

[a] Observed only at 30 mmol/L nucleoside concentration.
[b] Amount of the different complexes and their proposed models.

dispersed by ultrasonication, nanoaggregates with a mean hydrodynamic diameter of 10 nm were detected.

CD measurements were also performed to get further insights into the supramolecular organization of the hydrogel. Fig. 6 shows the CD spectra obtained for Sb(V)–G mixture at a 1:2 molar ratio following dilution in water at 1 mmol/L, just after heating for 1.5 h at 60 °C and after gel formation (cooling at room temperature). These spectra can be compared to those obtained for free G and Sb(V)–GMP mixture at a 1:2 molar ratio after heating and cooling at room temperature.

The spectrum for the Sb(V)–G mixture after heating (before gel formation) was very similar to that obtained for the Sb(V)–GMP mixture (after heating and cooling) that forms no gel. However, this spectrum differed markedly from that of free G, indicating that complex(es) was indeed formed. Strikingly, a couplet-type signal centered at 248 nm with two Cotton effects of opposite signs, a negative Cotton effect at 241 nm and a positive Cotton effect at 258 nm, was observed. Such a signal can be attributed to excitonic coupling between nucleobases that is expected to occur in the 1:2 Sb(V)–guanosine complex, as previously reported for a 1:2 Sb(V)–adenosine complex [8]. We can also infer that a right-handed screw conformation exists between the transition dipole moments corresponding to the $\pi \rightarrow \pi^*$ transition of the base; i.e., the chirality is positive. Cooling of the Sb(V)–G mixture induced further modifications of the CD spectrum, with the appearance of an intense couplet-type signal centered at 297 nm with a positive Cotton effect at 280 nm and a negative Cotton effect at 311 nm. This signal can be attributed to additional interactions between nucleobases involving excitonic coupling, most probably base stacking, also suggested by the 30% hypochromic effect (data not shown) [27]. At this second organization level, a left-handed screw conformation exists between the transition dipole moments; i.e., the chirality is negative.

From these data, we propose that the formation of Sb(V)–G hydrogel results from two types of interactions: (i) covalent bonds forming Sb(V)–G complexes and (ii)

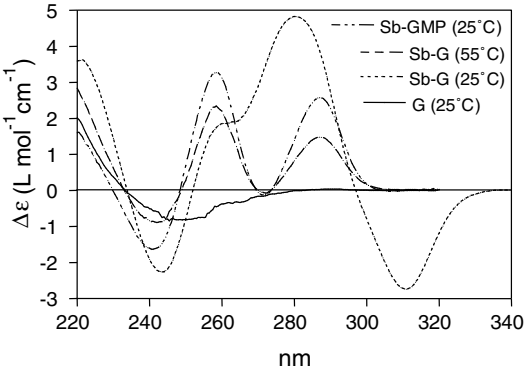


Fig. 6. CD spectra obtained for free G, for Sb(V)–G mixture at a 1:2 molar ratio, just after heating for 1.5 h at 60 °C and after cooling at room temperature and for Sb(V)–GMP mixture at a 1:2 molar ratio after heating and cooling at room temperature. Samples were diluted at 1 mmol/L for CD measurements.

intermolecular interactions between the different Sb(V)–G complexes via base stacking.

It has been reported that guanylic acids and guanine-rich oligonucleotides form liquid crystalline phases of both the cholesteric and hexagonal types in water [9]. The building blocks for these phases are columnar aggregates based on the G quartet. The G quartet is formed by the self-assembly of four guanine bases, a process that is templated by alkali metal ions such as K^+. In these systems, gel formation occurs using relatively high concentrations of alkali metal ions (typically 0.15 mol/L KCl). In the present system, the binding of Sb(V) to ribose but not to the nucleobase suggests that G quartets may be formed in the Sb(V)–G hydrogel. Strikingly, hydrogel formation occurs with K^+ concentrations as low as 5 mmol/L.

4. Conclusions

We report here the preparation and structural characterization of 1:1 and 1:2 Sb(V)–adenosine complexes isolated in the solid state. We show that Sb(V) can be pentacoordinated by two riboses and one hydroxyl or octacoordinated by two riboses and two hydroxyls or by one ribose and four hydroxyls. Furthermore, a 1:2 Sb(V)–guanosine thermosensitive and translucent hydrogel composed of a misture of 1:1 and 1:2 Sb(V)–guanosine complexes has also been obtained. Evidence for nanoassembling with two types of interaction: (i) covalent bonds through the formation of Sb(V)–guanosine complexes and (ii) intermolecular interactions between the different Sb(V)–guanosine complexes involving base stacking, have been presented. The formation of 1:1 Sb(V)–ribonucleoside complex at physiological pH is of great relevance, since it should help elucidating the mechanism of action of pentavalent antimonial drugs.

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