Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

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First published on: 17 February 2010

To cite this Article Fuentes-Martínez, Yanet , Godoy-Alcántar, Carolina , Medrano, Felipe , Dikiy, Alexander and Yatsimirsky, Anatoly K.(2010) 'Nucleotide recognition by protonated aminoglycosides', Supramolecular Chemistry, 22: 4, 212 — 220, First published on: 17 February 2010 (iFirst)
To link to this Article: DOI: 10.1080/10610270903254134
URL: http://dx.doi.org/10.1080/10610270903254134

Supramolecular Chemistry
Vol. 22, No. 4, April 2010, 212–220

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Nucleotide recognition by protonated aminoglycosides

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(*Received 2 April 2009; final version received 4 August 2009*)

Interactions of protonated forms of kanamycin A with nucleotides and several simple phosphate anions have been studied by potentiometric and NMR titrations. The affinity of kanamycin A to anions is comparable to that observed with other aliphatic polyammonium receptors of similar charge, but it discriminates triphosphate nucleotides with different nucleobases with binding constants following the order GTP $\gg$ CTP $\approx$ ATP. Kanamycin A also binds the respective uncharged nucleosides with the same selectivity. Binding of ATP is exothermic with a negative entropic contribution in contrast to what is expected for simple ion pairing. Other tested aminoglycosides, amikacin and streptomycin, bind ATP less efficiently than kanamycin A. Models of structures of kanamycin A complexes with ATP and GTP obtained by molecular mechanics (OPLS-2005) calculations based on ^{1}H and ^{31}P NMR data confirm the possibility of nucleotide discrimination by simultaneous ion pairing of terminal nucleotide phosphate groups with ammonium sites of rings B and C and hydrogen bonding of the nucleobase at the ring A of the aminoglycoside.

Keywords: binding constant; aminoglycosides; molecular recognition; nucleobases; potentiometry

1. Introduction

Protonated synthetic polyamines, both macrocyclic and acyclic, have been extensively studied as receptors for recognition of inorganic and organic anions including nucleotides (*1–3*). Aminoglycosides are natural polyamines (*4*), which in their protonated forms may have rather unique properties as anion receptors since they possess high total positive charges with ammonium groups arranged in a specific pattern over a relatively rigid scaffold. They also have chiral centres and may be suitable for enantioselective recognition of chiral anions. Indeed, fradiomycin, kanamycin and streptomycin were employed as chiral selectors in capillary electrophoresis (*5*). Preliminary results on binding of some organic and inorganic anions to protonated forms of neomycin B have been published emphasising a potential utility of aminoglycosides for anion recognition (*6*); however, this aspect has been never studied in depth.

The main purpose of this paper was to evaluate the affinity and selectivity of anion recognition by protonated forms of aminoglycosides. We choose for this study, kanamycin A (*7*), a relatively inexpensive aminoglycoside, which is commercially available as sulphate in sufficiently pure form. The crystal structure (*8*), complete assignment of NMR spectra (*9*), solution properties such as protonation (*10*) and conformational equilibria (*11*), has been reported for this aminoglycoside, creating a solid basis for the interpretation of its recognition properties. For comparative purposes, some measurements were also performed with two other aminoglycosides, i.e. amikacin and streptomycin (Scheme 1).

In preliminary experiments, we found that kanamycin A bound mono- and poly-phosphate anions and could discriminate triphosphate nucleotides with different nucleobases. Therefore, the principal emphasis was made on recognition of nucleotides. Currently, much attention has been paid to the nucleobase-directed selectivity in nucleotide recognition (*12*). A general approach is to incorporate into cationic receptor aromatic fragments, which may recognise the nucleotide base by stacking interactions, while sufficiently high affinity is ensured by electrostatic attraction to the positively charged receptor (*13*). Our results demonstrate that similar efficiency of nucleobase recognition can be achieved with purely aliphatic receptors due to strong enough interactions of nucleobases with the carbohydrate moiety.

2. Results and discussion

2.1 Potentiometry

The association constants of anions with differently protonated forms of aminoglycosides were determined by potentiometric titrations of their mixtures (typically 2 mM) and sodium salts of anions applied in the range 2–6 mM in

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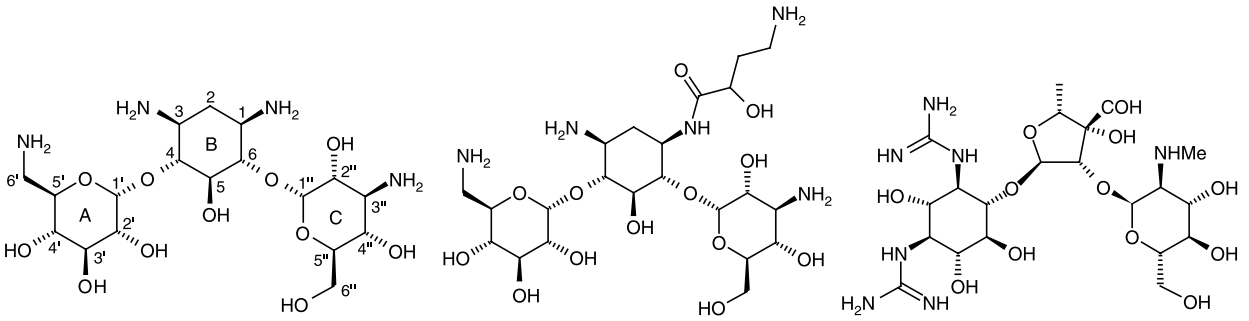
ISSN 1061-0278 print/ISSN 1029-0478 online

DOI: 10.1080/10610270903254134
http://www.informaworld.com



Scheme 1.

the presence of 0.1 M NaCl as a background electrolyte. The fitting of the titration curves allows one to determine the overall stability constants defined by Equation (1), which correspond to reaction (2) (charges omitted):

$$\beta_{11n} = [\mathrm{LAH}_n]/[\mathrm{L}][\mathrm{A}][\mathrm{H}]^n, \quad (1)$$

$$\mathrm{L} + \mathrm{A} + n\mathrm{H} \rightleftarrows \mathrm{LAH}_n. \quad (2)$$

In these equations, L and A stand for completely unprotonated forms of aminoglycoside and the anion, respectively. Then, the stepwise association constants K_{ij}, which correspond to complex formation between individual ionic forms of receptor (LH_i) and anion (AH_j), where $i + j = n$, can be calculated from β_{11n} values with known protonation constants of both reactants.

Testing interactions of kanamycin A with carboxylates revealed only weak binding of anions of mono- and dicarboxylic acids. Binding of the trianion of citrate was detectable with $\log K = 2.79$ for association with tetraprotonated kanamycin A. Similar affinity ($\log K = 2.7$) was reported for binding of citrate trianion with a tetraprotonated polyammonium macrocyclic receptor (*14*). Higher affinities, however, were observed with mono- and poly-phosphate anions.

Logarithms of protonation constants of aminoglycosides and anions and of stepwise formation constants of their complexes are given in Tables 1–3. Logarithms of the respective overall protonation and stability constants are shown in Tables S1–S3 (Supplementary Material).

As follows from Table 2, kanamycin A binds dianions of inorganic phosphate and phenylphosphate with similar strength. Association constants for the tetraprotonated receptor are close to those reported for linear acyclic (*15*) and macrocyclic (*16*) tetraprotonated polyamines. Formally calculated association constants for the tetra-anion of pyrophosphate (Table 2, constants given in parentheses) are very large, but this anion is strongly basic with first protonation constant larger than the second protonation constant of kanamycin A. Assuming the monoprotonated anion to be the real guest, one obtains smaller association constants for the binding of $HP_2O_7^{3-}$ in the range of $\log K$ from 2.9 to 3.6, again similar to those reported for acyclic polyamines (*15*).

The association with nucleotides is significantly stronger. Already AMP, which is a dianion of basicity similar to that of phenyl phosphate and hydrogen phosphate, forms notably more stable complexes with similarly charged forms of kanamycin A and also forms a detectable complex with monoprotonated receptor. Interactions with ADP and ATP are progressively stronger in line with increased negative charge of the guest. Association constants for CTP practically coincide with those for ATP, but the binding of GTP is much stronger. This nucleotide also has a significant affinity to the neutral form of kanamycin A.

The really observed affinity of a receptor to the guest under given conditions is expressed by an observed binding constant (K_{obs}) defined in terms of total concentrations of free reactants and the complex (*1a*). The discussion of binding selectivity of kanamycin A towards different phosphate guests will be clearer with using of such constants, which can be easily calculated from the overall β values with Equation (3):

$$K_{\mathrm{obs}} = \frac{\sum[\mathrm{LAH}_i]}{\left(\sum[\mathrm{LH}_j]\right)\left(\sum[\mathrm{AH}_k]\right)} = \frac{\beta_{110} + \beta_{111}[\mathrm{H}^+] + \beta_{112}[\mathrm{H}^+]^2 + \beta_{113}[\mathrm{H}^+]^3 + \cdots}{(1+\beta_{101}[\mathrm{H}^+]+\beta_{102}[\mathrm{H}^+]^2+\cdots)(1+\beta_{011}[\mathrm{H}^+]+\beta_{012}[\mathrm{H}^+]^2+\cdots)}. \quad (3)$$

Figure 1 shows $\log K_{\mathrm{obs}}$ vs. pH profiles for adenine nucleotides, GTP and inorganic phosphate ions. The strongest binding with all guests is observed in the pH range 6–7 where kanamycin A exists predominantly as a mixture of tri- and tetra-protonated forms. At lower pH phosphate, guests become increasingly more protonated and, at higher pH, kanamycin A becomes deprotonated. In this range of pH, the difference in the K_{obs} values for GTP and ATP is one order of magnitude, although the

Table 1. Logarithms of the stepwise protonation constants of aminoglycosides, nucleotides, nucleosides and phosphate anions at 25°C in 0.1 M NaCl.[a]

Reaction[b]	**I**[c]	**II**[c]	**III**[c]	AMP	ADP	ATP	CTP	GTP	PhP[d]	HPO_4^{2-}	$P_2O_7^{4-}$	Ado	Guo
H+X = HX	9.16 (2)	9.78 (4)	7.94 (1)	6.28 (2)	6.44 (2)	6.57 (2)	6.75 (3)	9.74 (4)	5.90 (3)	6.77 (3)	8.41 (4)	3.59 (5)	9.59 (4)
H+HX = H_2X	8.27 (2)	8.91 (4)		4.03 (6)	3.97 (6)	4.03 (5)	4.69 (6)	6.53 (6)			6.01 (6)		2.97 (6)
H+H_2X = H_3X	7.52 (4)	7.78 (4)						3.91 (7)					
H+H_3X = H_4X	6.28 (8)	6.88 (4)											

[a] Values in parentheses are standard errors in the last significant digit.
[b] X = L or A in the deprotonated form; charges omitted.
[c] **I**, kanamycin A; **II**, amikacin; **III**, streptomycin.
[d] PhP = $PhOPO_3^{2-}$.

maximum discrimination between them with $\Delta\log K_{obs} = 1.2$ is observed at somewhat lower pH 5. The discrimination between ATP, ADP and AMP is principally by charge and stronger binding of ATP as compared to inorganic phosphate can be attributed to the interaction with nucleobase (see below). Surprisingly small discrimination is seen between ATP and pyrophosphate, which at pH below 7 has a smaller by unity total charge and lacks any organic moiety. Apparently, this anion fits particularly well to the ammonium groups of protonated kanamycin A.

Binding of ATP was studied in more detail. The binding constants of ATP to protonated forms of amikacin (Table 3) are very close to the respective values for kanamycin A (Table 2). These antibiotics have similar structures and the same number of amino groups with the amino group at C1 in ring B of kanamycin A substituted with a (*S*)-4-amino-2-hydroxybutyryl group in amikacin (Scheme 1). Interestingly, the binding to streptomycin is essentially charge-independent (Table 3), indicating possibly even higher contribution of non-electrostatic interactions in this case.

Stepwise association constants with ATP were determinated at different temperatures (Table 4) and analysed by the van't Hoff method to calculate the thermodynamic parameters (Table 5) for the binding of ATP with different protonated forms of kanamycin A.

Although calculated values are rather approximate, they clearly show that the association is always exothermic with negative entropic contribution. Purely electrostatic ion pairing is typically entropy driven and is characterised by positive $T\Delta S$ and close to zero or positive ΔH. Such thermodynamic characteristics were reported for ATP binding to linear (*17*) and cyclic (*18*) polyammonium receptors. On the other hand, both the enthalpy- and entropy-driven associations of phosphate and pyrophosphate anions with polyamines were reported and the exothermic contribution was attributed to hydrogen bonding between neutral amino groups of receptors and OH groups of partially protonated anions (*15*). This type of interaction is not expected, however, for any of the reactions in Table 5. More plausible explanation is a significant contribution of non-electrostatic interactions between ATP and the aminoglycoside. Binding of ATP to proteins, which involves a significant contribution of such interactions, is exothermic with ΔH values about -70 kJ/mol (*19*).

2.2 NMR spectroscopy

Chemical shifts of ^{31}P signals of ATP and GTP in the absence and presence of kanamycin A as a function of pH are shown in Figure 2. As is observed with other protonated polyamines (*2c*, *3f*, *3g*, *20*) addition of kanamycin A induces downfield shifts of the signals, the largest one for the terminal γ-phosphate group and the

Table 2. Logarithms of the stepwise association constants of anions (A) with kanamycin A (L) at 25°C in 0.1 M NaCl.[a]

Reaction	HPO_4^{2-} ($n=2$)	$PhOPO_3^{2-}$ ($n=2$)	$P_2O_7^{4-}$ ($n=4$)	AMP^{2-} ($n=2$)	ADP^{3-} ($n=3$)	ATP^{4-} ($n=4$)	CTP^{4-} ($n=4$)	$HGTP^{4-}$ ($n=4$)	Ado ($n=0$)	Guo ($n=0$)
$L+A^{n-}$								2.45		3.12
$LH^{+}+A^{n-}$			2.3	1.75	2.35	1.90	1.86	2.60	2.28	3.04
$LH_2^{2+}+A^{n-}$	1.10		(3.1)	1.86	2.51	2.44	2.34	3.16	2.37	3.41
$LH_3^{3+}+A^{n-}$	1.90	1.76	(4.0)	2.20	3.02	3.20	3.32	3.76	2.46	3.49
$LH_4^{4+}+A^{n-}$	2.09	2.16	(5.3)	2.47	3.40	4.15	4.26	5.05	2.29	3.35
$LH^{+}+AH^{1-n}$			2.9							
$LH_2^{2+}+AH^{1-n}$			3.1							
$LH_3^{3+}+AH^{1-n}$	1.60	2.44	3.1	2.47	3.10	3.86	3.79	4.80		
$LH_4^{4+}+AH^{1-n}$			3.6		1.84	3.00	3.04			
$LH_3^{3+}+AH_2^{2-n}$			3.8							
$LH_4^{4+}+AH_2^{2-n}$			2.6			2.90	2.84	4.32		

[a] Errors ±0.02–0.1, inorganic phosphates; ±0.02–0.06, nucleotides; ±0.02–0.08, nucleosides.

Table 3. Logarithms of the stepwise association constants of ATP (A) with amikacin and streptomycin (L).[a]

Reaction	Amikacin	Streptomycin
$LH^{+}+A^{4-}$	1.93 (4)	
$LH_2^{2+}+A^{4-}$	2.11 (2)	
$LH_3^{3+}+A^{4-}$	3.18 (2)	2.77 (3)
$LH_4^{4+}+A^{4-}$	3.93 (2)	
$LH_3^{3+}+AH^{3-}$	4.25 (4)	2.33 (4)
$LH_4^{4+}+AH^{3-}$	2.90 (5)	
$LH_3^{4+}+AH_2^{2-}$		2.65 (4)
$LH_4^{4+}+AH_2^{2-}$	2.58 (7)	

[a] Values in parentheses are standard errors in the last significant digit.

Table 4. Logarithms of the overall and stepwise stability constants for the binding of ATP to kanamycin A at different temperatures.

Reaction	15°C	25°C	35°C
L+A+H	10.82 (4)	10.63 (5)	10.25 (6)
L+A+2H	20.26 (3)	19.59 (4)	19.25 (4)
L+A+3H	28.88 (3)	28.08 (3)	27.59 (3)
L+A+4H	36.50 (2)	35.34 (3)	34.74 (3)
L+A+5H	41.76 (4)	40.65 (5)	40.07 (5)
LH+A	1.41 (8)	1.5 (1)	1.3 (1)
LH_2+A	2.41 (9)	2.16 (8)	2.07 (9)
LH_3+A	3.30 (7)	3.13 (8)	3.06 (8)
LH_4+ A	4.47 (9)	4.11 (7)	4.14 (8)
LH_4+AH	3.15 (9)	2.86 (9)	2.75 (9)

smallest one for the α-phosphate group. The absolute values of $\Delta\delta$ are similar to those observed for purely aliphatic polyammonium macrocycles. The exact nature of these shifts has not been established yet, but it was suggested that major contributions are the change in the protonation degree of the nucleotide and possible conformation changes upon complexation (*3f*).

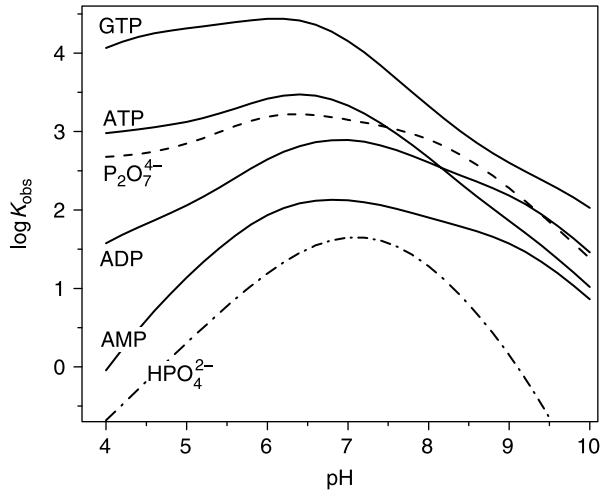


Figure 1. Calculated log K_{obs} for the binding of some guests with kanamycin A at variable pH.

Figure 3 illustrates the distribution of ATP–kanamycin A complexes as a function of pH under conditions of the NMR experiment. The degree of complexation of ATP is between 70 and 80% in the pH range 5–8; it decreases to 45% at pH 9 and to 15% at pH 10. In the case of GTP, similar calculations show that the complexation is nearly quantitative at pH below 8, decreases to 70% at pH 9 and to 65% at pH 10. Thus, with both nucleotides, the degree of complexation at pH above 8 is still significant but the interaction with kanamycin induces very small if any shifts in signals. In this range of pH, phosphate groups of free nucleotides are deprotonated, but at pH 7 they are *c*. 30% protonated and at pH 5 nucleotides exist principally in mono-protonated forms. This protonation induces typical upfield shifts of ^{31}P signals, which are partially compensated by addition of kanamycin due to smaller degree of protonation of nucleotides in complexes with the cationic receptor. Thus, the major contribution to observed downfield shifts is from the change in the protonation degree of the nucleotide.

Table 5. Thermodynamic parameters (kJ/mol) for the association of ATP (A) with differently protonated forms of kanamycin A (L).

	ΔH°	$T\Delta S^\circ$	$\Delta G^{\circ a}$
$LH^{+}+A^{4-}$	-12 ± 18	-4 ± 6	-8.7
$LH_2^{2+}+A^{4-}$	-29 ± 7	-17 ± 5	-12.3
$LH_3^{3+}+A^{4-}$	-20 ± 4	-3 ± 1	-17.8
$LH_4^{4+}+A^{4-}$	-28 ± 8	-5 ± 2	-23.4
$LH_4^{4+}+AH^{3-}$	-34 ± 8	-18 ± 5	-16.3

[a] At 25°C; standard errors ± 0.3 kJ/mol.

On this assumption, the results in Figure 2 can be used to analyse the possible proton transfer between anion and receptor inside complexes. Thus, the most abundant species, a neutral complex LAH_4, can be either a complex between LH_4^{4+} and A^{4-} or a complex between LH_3^{3+} and AH^{3-}, with $\log K$ values of 4.15 and 3.86, respectively (Table 2). The protonation constant of ATP is larger than the fourth protonation constant of kanamycin A by $\Delta\log K = 0.29$ (Table 1) and the intermolecular proton transfer from AH^{3-} to LH_3^{3+} would be accompanied with a positive free energy change of 1.5 kJ/mol. On this basis, the preferable complexation is between LH_3^{3+} and AH^{3-}, but the free energy gain from the formation of an additional salt bridge in the complex between LH_4^{4+} and A^{4-} typically of -2.5 kJ/mol (*21*) would be sufficient to overcome this free energy loss. From the results in Figure 2(A), one observes that at pH 7 when 50% of ATP are in the form of LAH_4 (Figure 3) chemical shifts of γ- and β-phosphate groups correspond to less than 10% degree of protonation of ATP, indicating that the bound nucleotide is mostly the tetra-anion.

Figure 4 shows the changes in ^{1}H chemical shifts of kanamycin A on addition of various guests. The largest (downfield) shifts with all guests apart from AMP are observed for protons of the methylene group at C2 in the ring B, situated between two ammonium groups. They can be attributed to the deshielding effect of the phosphate group (see below). Inorganic phosphates also significantly affect the shift of C1′ proton in the ring A. All nucleotides induce upfield shifts in the ring A possibly due to the shielding effect of the aromatic system of the nucleobase. The behaviour of AMP is different in the sense that it affects very little C2 protons and induces significant upfield shifts in the ring C as well as in signals of C5 and C6 in the ring B closest to the ring C. The latter may reflect the existence of two binding modes for AMP with the nucleobase bound to either ring A or ring C, which are in a fast equilibrium with each other. At the same time, it seems that the ion pairing with this nucleotide is less significant. This agrees with relatively weak dependence of the binding constant for AMP^{2-} on the total kanamycin charge (see Table 2). Comparing the complexation-induced shifts for ATP and GTP, one observes larger shifts for the former in spite of stronger binding of the latter. Such 'inversed' correlation between the affinity and complexation-induced ^{1}H NMR shifts was also observed for the binding of nucleotide triphosphates to a peptide receptor, and it does not have any simple interpretation (*22*).

Signals of nucleobase protons also underwent the complexation-induced shifts with $\Delta\delta$ 0.041 and 0.025 ppm for protons at C8 and C1′ for GTP and -0.028 for protons C2 and C8 and -0.018 ppm for C1′ of ATP. Similar absolute value shifts in both directions were also observed on complexation of nucleotides with aminocyclodextrins (*23*).

The above results as well as the very fact of different stabilities of complexes with ATP and GTP point to the possible interaction of kanamycin A with the nucleobase.

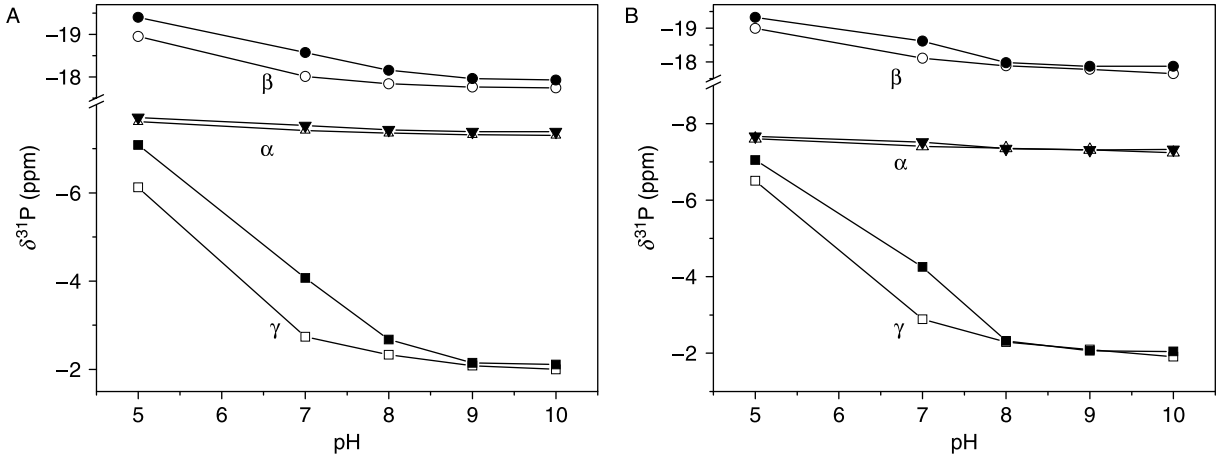


Figure 2. Chemical shifts of ^{31}P as a function of pH for (A) 0.02 M ATP and 0.02 M kanamycin-ATP and (B) 0.02 M GTP and 0.02 M kanamycin-GTP. Solid symbols refer to free nucleotide, open symbols to the mixture with kanamycin, triangles, circles and squares refer to α-, β- and γ-phosphate groups, respectively.

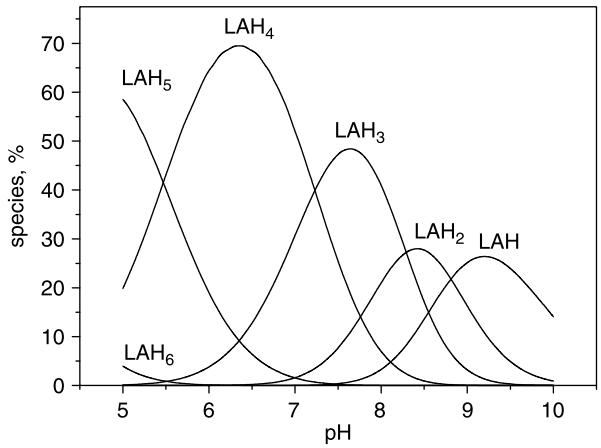


Figure 3. Distribution of complexes in an equimolar mixture of 0.02 M ATP (A) and kanamycin A (L) calculated with HYSS 2000 program.

To test this, we performed potentiometric titrations of kanamycin A in the presence of the corresponding nucleosides lacking the negative charge (Tables S2 and 2). The results indicate a fairly strong charge-independent association with the binding constant for guanosine, which is one order of magnitude larger than that for adenosine in agreement with stronger binding of GTP than ATP. Previously, a weak association of adenosine and cytidine with aliphatic polyammonium cations was reported (*24*). In fact, the comparison of logarithms of the binding constants of kanamycin A to ATP and GTP with those to adenosine and guanosine, respectively, shows that about half of the binding free energy of nucleotides can be attributed to non-electrostatic interactions, probably of the same origin as those which are responsible for the binding of carbohydrates by proteins (*25*) and which provide at least half of the free energy of binding of aminoglycosides to RNA and other polynucleotides (*26*).

2.3 Computational chemistry studies

Possible structures of complexes formed by tetraprotonated kanamycin A with ATP and GTP tetra-anions were generated by molecular mechanics with OPLS force field calculations. On the basis of NMR data, the nucleotide was initially positioned in front of the rings A and B of kanamycin A in either the *syn–syn* or *anti–syn* conformation. During subsequent minimisation, the nucleotide in complexes with the *anti–syn* conformer moved towards ring C leaving the ring A free of any close contacts with the nucleotide in disagreement with the NMR results. In addition, the calculated energy differences between free components and the complexes were less favourable by 20 and 40 kJ/mol for ATP and GTP, respectively, than those for the corresponding complexes with the *syn–syn* conformer. The minimised structures of complexes are shown in Figure 5. Calculated steric energies of complexes with ATP and GTP are −102.6 and −141.9 kJ/mol, respectively, confirming the higher stability of the GTP complex.

The principal attractive interactions in the GTP complex in accordance with the calculated structure (Figure 5(A)) are remarkably similar to those revealed from the X-ray crystal structure of the complex between the tetra-anion of TTP and a tetraprotonated polyammonium receptor containing terpyridine fragments (*13a*). In both cases, only two of four ammonium groups of the receptor participate in interaction with two phosphate groups of the nucleotide forming three ionic hydrogen bonds and there is one hydrogen bond between carbonyl oxygen of the nucleobase and one ammonium group of the ligand. In the complex with ATP, ionic interactions are the same, also in both ATP and GTP complexes, there is a close contact between the ammonium group at C3 position of kanamycin A and N7 atom of the nucleobase, which may provide some attractive ion–dipole interaction, but in the complex with ATP C6′ ammonium group interacts with

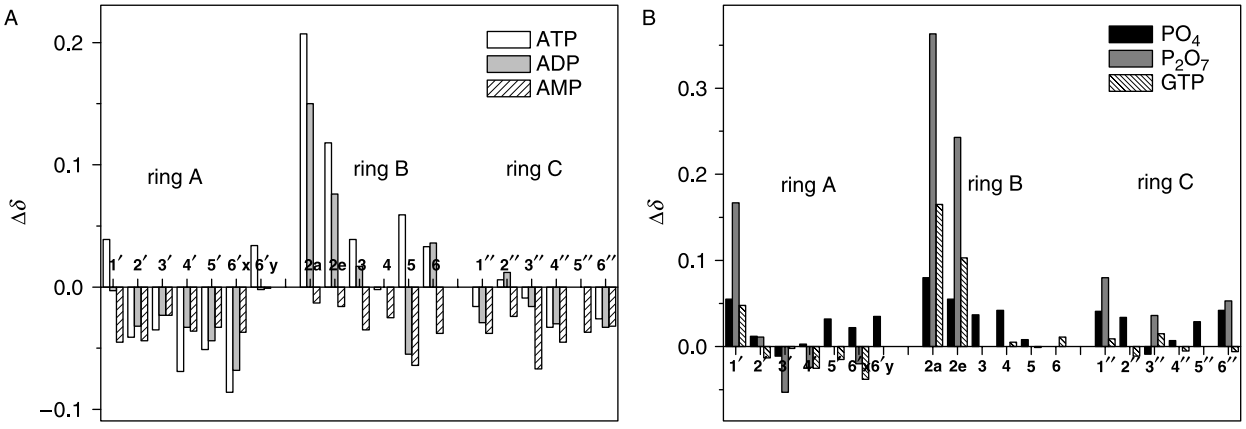


Figure 4. Changes in ^{1}H chemical shifts of the 0.02 M kanamycin A on addition of 0.02 M guests at pH 7. (A) Adenine nucleotides and (B) GTP and inorganic phosphates.

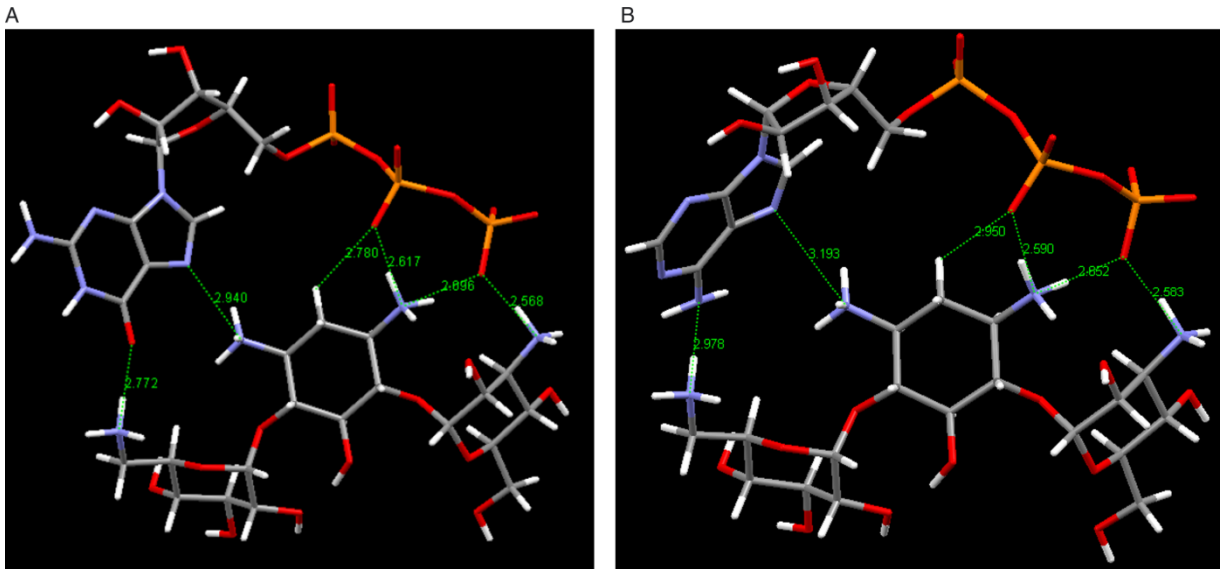


Figure 5. Simulated structures of the complexes formed by tetraprotonated kanamycin A and tetra-anions of GTP (A) or ATP (B).

a much less basic amino group of adenine instead of the carbonyl group of guanine and this explains the lower stability of the ATP complex. In both complexes, there is a short contact between the β-phosphate group of the nucleotide and C2 methylene in the ring B of kanamycin A, which may be the reason for a significant complexation-induced downfield shift of the signals of protons of this group (Figure 4).

3. Conclusions

In conclusion, we would like to compare the binding affinity and selectivity of tetraprotonated kanamycin A with those for some recently reported cationic receptors specially designed for nucleotide recognition (Table 6).

All receptors in Table 6 have the same total charge 4+ and should provide similar electrostatic contribution to the binding of nucleotide anions. Cleft type and macrocyclic receptors **1**–**5** contain aromatic fragments set aside for recognition of the nucleobase via stacking interactions. However, they demonstrate to be similar to kanamycin A in the degree of discrimination of ATP and GTP but with high total affinity in the case of receptor **2**. Peptide **6** with three lysyl and one arginyl residue has much smaller total affinity and discrimination capacity, the latter attributed to stacking interactions with tryptophanyl residues. Finally, a highly affine receptor **7**, which is a binuclear Zn(II) complex with a neutral pyridine-type ligand, does not discriminate even nucleotide di- and triphosphates.

Thus, the results obtained in this study with kanamycin A demonstrate that aminoglycosides are promising nucleotide receptors, which do not require a laborious synthetic preparation and are similar to other specially designed receptors by their affinity and selectivity. An obvious disadvantage of simple aminoglycosides as receptors is the absence of an easily detectable optical or any other signal accompanying the recognition process. We expect to solve this problem in further studies by attaching a chromogenic group to an aminoglycoside. We

Table 6. Logarithms of the binding constants of nucleotides with some tetracationic receptors (for chemical structures of receptors see Scheme S1 in the Supplementary Material).

	log *K*					
Receptor	AMP	ADP	ATP	CTP	GTP	Ref.
Kanamycin A	2.47	3.41	4.15	4.26	5.05	This work
Bis-(imidazolylmethyl)anthracene cleft, **1**	2.08	2.79	4.18		4.94	(*13c*)
Phenanthroline polyammonium macrocycle, **2**	4.25	4.91	7.08	5.98	5.75	(*21*)
Cavitand, **3**			4.15	3.89	4.87	(*12e*)
Pyrenophane, **4**	3.28	3.72	6.00	5.41	6.11	(*27*)
Cyclo-bis-intercaland, **5**	4.2	4.4	5.4		6.8	(*28*)
β-Hairpin peptide, **6**			2.84	2.43	3.34	(*22*)
Binuclear Zn(II) complex, **7**		6.23	6.11	5.83	6.23	(*12f*)

also believe that a study of aminoglycoside–nucleotide interactions may be useful for a better understanding of interactions of aminoglycoside antibiotics with their target polynucleotides.

4. Materials and methods

4.1 Materials

All reagents were purchased from Aldrich and used as received. The purity of aminoglycosides was confirmed by NMR spectra and potentiometric titrations.

4.2 Potentiometry

Potentiometric titrations were performed in a 250 ml thermostated cell kept under nitrogen at desired temperature, typically 25 ± 0.1°C using 0.1 M NaCl as the supporting electrolyte. Measurements of pH were carried out using a Thermo Orion model 920A-plus pH meter equipped with an Orion 8102U combination electrode while the titrant (CO_2-free NaOH) solution was added to the system in small increments with a piston-type burette. The glass electrode was calibrated as a hydrogen-ion concentration probe by titration of previously standardised amounts of HCl with CO_2-free NaOH solutions. The equivalent point was determined by Gran's method, which gives the ionic product of water ($pK_w = -13.98$). The details of the electrode calibration were described previously (*29*). The program HYPERQUAD 2003, version 3.0.51 (*30*), was used to calculate all equilibrium constants. Species distribution diagrams were calculated using HYSS 2000 software (*30b*). The pH range investigated was 3–10.5, the concentration of guests from 0.02 to 0.06 M and kanamycin A was 0.02 M.

For the determination of the thermodynamic parameters, potentiometric titrations of kanamycin A, ATP and their mixtures were performed at 15, 25 and 35°C with temperature compensation of the pH meter in triplicate to obtain protonation and overall formation constants at each temperature from which the stepwise association constants were calculated as described in the text. Then, $\Delta H°$ values were calculated from the slopes of the plots of $\ln K$ vs. $1/T$ for each association constant assuming $\Delta c_P = 0$. The $T\Delta S°$ values were calculated with the van't Hoff equation from the $\Delta G°$ values at 25°C and respective $\Delta H°$.

4.3 NMR spectra

All the ^{31}P NMR spectra were recorded at 80 MHz on a Varian MERCURY spectrometer (200 MHz). Chemical shifts are relative to an external reference of triphenylphosphate ($\delta = -16.6$ ppm). The ^{1}H NMR spectra were recorded on a Varian UNITY INOVA spectrometer (400 MHz). The spectra were obtained at room temperature in D_2O solutions and pH was adjusted to the desired value with DCl or NaOD in the range of 5–10. For ^{1}H NMR, the solvent signal was used as a reference standard.

4.4 Computational method

The structures of kanamycin A–nucleotide complexes were calculated by molecular mechanics with the OPLS-2005 force field (*31*) and Polak–Ribiere conjugate gradient minimisation scheme. All calculations were performed in water using a generalised Born/surface area continuum solvation model (*32*) as implemented in MACROMODEL (*33*).

Acknowledgements

This work was supported by CONACyT, project 60747; YFM thanks CONACyT for the PhD Fellowship.

Supplementary Material

Overall stability constants for the binding of ATP to kanamycin A at different temperatures and chemical structures of receptors are listed in Table 6, available online.

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