Anion coordination by aminoglycosides: structural and charge effects

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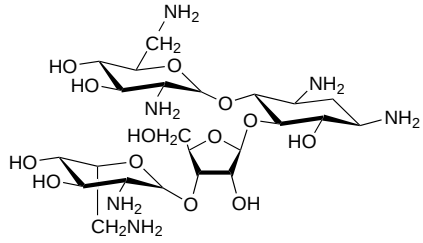
Aminoglycosides provide new approaches to the recognition and specific binding of anionic species, and show promise for development as anion sensors in biological and separation science.

Aminoglycoside antibiotics constitute a large family of molecules that find extensive clinical use in the treatment of gram-negative infections.[1] Recent reports have also demonstrated specific and high affinity (μM) binding of aminoglycosides to RNA structural motifs.[2–6] Aminoglycoside complexes of metal ions have previously been reported,[7–10] however, the large positive charge density of the drug in neutral solution suggested to us that such molecules might prove to be efficient anion complexing agents. This work contrasts with earlier studies of anion binding that have focused on polyamine macrocycles including hexaaza ligands and porphyrin derivatives,[11–14] Novel advances in this chemistry should afford significant opportunities for the development of anion sensors,[15–17] and in separation science.[18–20] Here, we communicate our initial findings on the coordination chemistry of neomycin B **1** with a variety of negatively charged species. Binding thermodynamics have been evaluated by isothermal titration calorimetry (Fig. 1), and structural insight obtained by ^{1}H, ^{13}C, ^{15}N and ^{31}P NMR spectroscopy.‡ These studies complement recent work on ferrocenyl receptors of anions.[16] In contrast to the classic inner-sphere coordination chemistry of metal ions, anion binding more typically involves outer-sphere binding of multi-atomic species; although outer-sphere interactions of cationic complexes are also well established in biological chemistry.[21–24]

Neomycin B, **1**, possesses six ionizable amino functional groups, with pK_as > 6.5, and it was expected that such a highly charged molecule might complex anionic species with moderate to high affinity. Fig. 1 establishes this fact with K_a varying from 332 dm^3 mol^{-1} for CrO_4^{2-} to 1.3 × 10^5 dm^3 mol^{-1} for



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$Fe(CN)_6^{4-}$. The coordination chemistry of neomycin B has been explored with two classes of anionic species, differing in their distribution of charge density and described as spherical [$Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$, $Cr(C_2O_4)_3^{3-}$, CrO_4^{2-}], where the charge is confined in a spherical array, and linear [AMPH$^-$, ADPH^{2-}, ATPH^{3-}, and adenosine tetraphosphate (AtetraPH^{4-})], where the charge is spread along a chain of atoms. Distinct thermodynamic data (Fig. 1) were obtained for the spherical and linear charged species. Also the binding affinity is found to increase with increasing charge on both the aminoglycoside and bound anion, although different factors control the experimental K_as for each class of anion. The linear adenosine phosphate series demonstrates an approximately constant binding enthalpy [ΔH *ca.* −6.6 kcal mol^{-1} (1 cal = 4.184 J), Fig. 1], with the charge dependence of the binding affinity arising through variation of the entropy term, presumably as a result of more extensive disruption of the solvation state for the longer phosphate chain. In contrast, the spherical charged species show a marked variation for both the entropy and enthalpy terms; although for both spherical and linear charged species the magnitude of the ΔH term demonstrates that binding is enthalpically controlled. This contrasts with binding to oligo- or poly-nucleotides where ligand binding is typically entropically driven.

Our attention was drawn to the mode of binding of the anions to the aminoglycosides. None of the anion–aminoglycoside complexes demonstrate the direct inner-sphere binding mode typical of classical metal ion coordination to a basic ligand. Rather, outer-sphere binding must be mediated either through hydrogen bonding and/or electrostatic interactions. The similarity in binding affinity for ATPH^{3-}, $Fe(CN)_6^{3-}$ and $Cr(C_2O_4)_3^{3-}$, and their very different hydrogen-bond accepting abilities (decreasing across the series), suggest the dominance of electrostatic attraction in defining K_a. Further support for this conclusion came from the salt dependence of K_a. In the Debye concentration range, the linear dependence of ln K_a with [NaCl]$^{1/2}$, where NaCl is the background electrolyte, is consistent with outer-sphere complex formation and long-range electrostatic interactions.[25] Plots of ln K_a *vs.* [NaCl]$^{1/2}$ for $Fe(CN)_6^{4-}$, ATPH^{3-} and CrO_4^{2-} binding to neomycin B were fitted to the equation, $\ln K_a = \ln\{(4\pi N_L a^3)/3000\} + b - ab\{(2Z^2e^2)/(\varepsilon\varepsilon_0 kT)\}^{1/2}[\mathrm{NaCl}]^{1/2}$, where symbols are defined in ref. 25. In each case linearity was maintained in the Debye range, and gradients of −5.7, −11.9 and −9.4, were obtained, respectively.

Structural aspects of anion binding to neomycin B have been evaluated by heteronuclear 1D and 2D NMR methods.

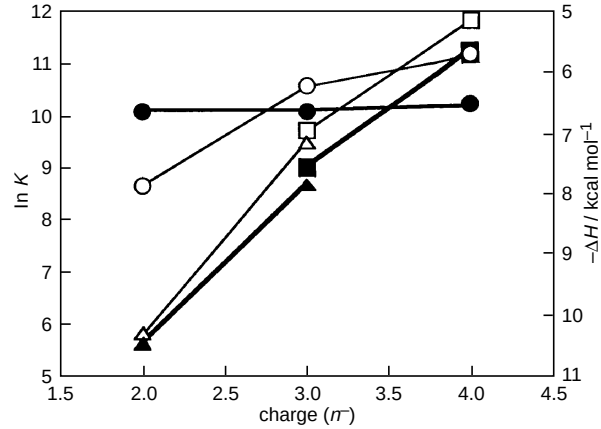
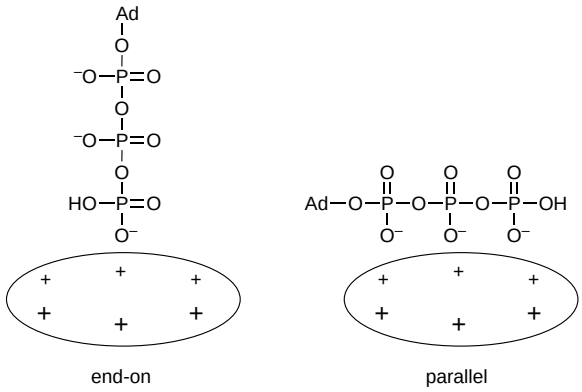


Fig. 1 Variation of thermodynamic binding parameters with the total charge of the anionic species (*n*). ADPH^{2-}, ATPH^{3-}, AtetraPH^{4-} (circles); $Fe(CN)_6^{3-/4-}$ (squares); CrO_4^{2-}, $Cr(C_2O_4)_3^{3-}$ (triangles), where open and closed symbols refer to ln *K* and ΔH data, respectively. Data were collected on a Microcal OMEGA isothermal titration calorimeter at pH 5.5 in 10 mM sodium acetate. No evidence for irreversible or covalent interactions was noted with these complexation reactions. Errors are within the radius of the symbols.

Assignments for ^{1}H, ^{13}C and ^{15}N resonances of neomycin have been previously reported.[26,27] Neither ^{1}H, ^{13}C, nor ^{15}N resonances or crosspeaks in 1D or 2D NMR experiments, respectively, demonstrated a significant change after binding of any of the anionic species to neomycin B, and so complex formation does not involve significant structural perturbation of the sugar rings. Fast exchange is indicated by the occurrence of only sharp exchange-averaged resonances for bound and free forms. ^{31}P NMR spectra of $ADPH^{2-}$, $ATPH^{3-}$ and $AtetraPH^{4-}$ show significant changes in the shift value of only the terminal phosphate upon binding to neomycin B. Also double protonation of the terminal phosphate at ATP at reduced pH (= 5) resulted in loss of binding, even though the overall charges of $ADPH^{2-}$ and $ATPH_2^{2-}$ are identical. These results support an end-on binding mode rather than a parallel mode (below). Such a hypothesis is also consistent with the approximately constant ΔH for binding of the adenosine phosphates (Fig. 1) to neomycin B, since only the terminal phosphate serves as an hydrogen-bond acceptor.



In conclusion, aminoglycosides have been shown to bind a variety of negatively charged species with moderate to high affinities, and structural differentiation of charge arrays (spherical and linear) has been demonstrated. The critical need for such molecules as biological sensors[10,17] and in separation science,[18–20] will fuel further efforts in our laboratory.

Notes and References

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‡ ^{1}H, ^{13}C, ^{31}P and ^{15}N NMR spectra were recorded on a Bruker 300 MHz spectrometer. ^{15}N measurements were made in 10 mm sample tubes with 200 mM solutions of neomycin. Other experiments were performed in 5 mm tubes with 100 mM neomycin solutions. A 200 mM sodium acetate buffer solution (pH 5) was used with 10% D_2O for spin lock. Buffer concentrations were higher than for calorimetry experiments to accommodate the increased neomycin concentration. 2D C–H correlation spectra were recorded with a phase-sensitive DEPT polarization transfer pulse sequence.

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