Aminoglycoside (Neomycin) Preference Is for A-Form Nucleic Acids, Not Just RNA: Results from a Competition Dialysis Study

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Since the discovery of aminoglycosides by Selman Waksman more than 50 years ago,1 most attention has focused on their binding to RNA2 and recently various other RNA structures.3 RNA affinity and discrimination by aminoglycosides is modulated by the interplay of nonspecific electrostatic forces, which are critical for affinity, and few specific interactions.4 The flexible and polycationic nature of the aminoglycoside antibiotics allows them to preferentially bind to prokaryotic ribosomal RNA, but also allows binding to a variety of unrelated RNAs, group I introns, a hammerhead ribozyme, the RRE transcriptional activator region from HIV (which contains the binding site for the Rev protein), the 5′-untranslated region of thymidylate synthase targets for important enzymes such as ribonuclease H and reverse mRNA, a variety of RNA aptamers from in vitro selection, and human mRNAs.5 Aminoglycoside charge has been suggested to be a necessary evil, leading to increased affinity, at the price of increased promiscuity and inefficient cellular uptake.

Our previous work has shown the remarkable ability of neomycin and other aminoglycosides and conjugates to stabilize DNA, RNA, and hybrid triple helices.6–10 Neomycin was shown by us to induce the stabilization of DNA-RNA hybrid duplexes as well as hybrid triple helices.6 This significantly added to the number of nucleic acids (other than RNA) that aminoglycosides have been shown to target. A clear requirement then arose for a quantitative assay to determine the relative binding affinities for host triplex, duplex DNA, single-stranded (DNA/RNA), and other possible nucleic acid targets (tetraplex) for a given aminoglycoside ligand. Fortunately, a rapid technique has now been established by Chaires for this exact purpose, using a thermodynamically rigorous competitive equilibrium dialysis method that exploits therapeutically useful drug concentrations.11,12 In the assay, solutions of different nucleic acid structures (of identical concentration) are dialyzed simultaneously against a common solution of ligand using appropriately buffered conditions. After equilibration, the amount of ligand bound to each DNA is measured by spectrophotometry. More ligand accumulates in the dialysis tube containing the structural form of highest binding affinity and, because all of the DNA samples are in equilibrium upon careful analysis of the dialysis data. All three drugs showed comparable binding to one nucleic acid: calf thymus DNA with comparable binding to one nucleic acid: calf thymus DNA.

Figure 1. Competition dialysis results of neo-acridine (1 μM) with various nucleic acids; 180 μL of different nucleic acids (75 μM per monomeric unit of each polymer) were dialyzed with 400 mL of 1 μM neomycin-acridine in BPES buffer (6 mM Na2HPO4, 2 mM NaH2PO4, 1 mM Na2-EDTA, 185 mM NaCl, pH 7.0) solution for 72 h. Among comparable single-stranded, duplex, and triplex structures, maximum binding is always observed with the triplexes.

This seemingly promiscuous binding leads to a different picture upon careful analysis of the dialysis data. All three drugs showed comparable binding to one nucleic acid: calf thymus DNA (Supporting Information). Calf thymus DNA also represents a standard duplex DNA. This observation was used to replot the dialysis results to emphasize differences relative to that standard. These results are shown in Figure 2 and better illustrate the change in specificity of the different acridines toward different nucleic acids. While 9-aminoacridine and quinacrine show a clear preference for DNA triplex, neomycin-acridine conjugate binding to RNA triplex is much greater than that to DNA triplex and even better than the natural aminoglycoside RNA target: eubacterial 16S A-site.

Figure 2. Competition dialysis results of neo-acridine (1 μM) with calf thymus DNA, 16S A-site rRNA (27 nt), and calf thymus DNA. This observation was used to replot the dialysis results to emphasize differences relative to that standard.

Scheme 1. Structures of Neomycin, Aminoacridines, and the Neomycin-acridine Conjugate

Neomycin

9-aminoacridine

Neomycin-acridine conjugate

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still lower than that to the RNA triplex. RNA-DNA duplexes are even better targets than DNA homoduplexes, poly(dA)-poly(rU) hybrid duplex being comparable in binding to the tetraplexes. Our experiments with aminoglycoside natural products have shown no effect on stability of A-T rich duplex DNA (in the presence of salt), suggesting weaker nonproductive binding. Triplexes are then the targets of choice for neomycin. Neomycin-acridine shows a remarkable binding preference to RNA triplex that has previously not been observed. A big surprise, however, is the significant binding observed with the poly(dG)-poly(dC) duplex.

Neo-acridine binding to RNA triplex was then investigated by UV thermal melts, ITC, and viscometric and CD titrations (Supporting Information). Thermal denaturation in the presence of neo-acridine shows an increase in Tm of 3°C (unpublished results). Groove recognition of triplexes and tetraplexes has been an elusive feat, where such charged polyamine binding factors may be the key to opening this Pandora’s box.

To conclude, this work clearly suggests that aminoglycoside specificity (neomycin, in a high nM to low μM range) is for nucleic acid forms that show some features characteristic of an A-type conformation (RNA triplex, DNA-RNA hybrid duplex, RNA tetraplex), rather than for naturally occurring RNA. While this work does not question that aminoglycoside’s mechanism of drug action involves binding to rRNA, it does challenge, as a matter of biochemical principles, the common belief that aminoglycoside specificity is simply for duplex RNAs.

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Supporting Information Available: CD spectra, melting curves for triplex/duplex, ITC/viscometric titration plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References


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