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From triplex to B-form duplex stabilization: reversal of target selectivity by aminoglycoside dimers

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Abstract—Aminoglycosides have been shown to target A-form nucleic acids. Our work has previously shown that neomycin (and other aminoglycosides) bind and stabilize DNA/RNA triplexes and other A-form nucleic acids. We report herein the unexpected B-form duplex stabilization shown by aminoglycoside dimers (neomycin–neomycin and neomycin–tobramycin). The dimers are highly selective for AT rich duplexes and show high affinity ($K_a \sim 10^8\,M^{-1}$) as determined by isothermal titration calorimetry.

Aminoglycoside antibiotics have been at the forefront of antimicrobial therapy for over half a century,[1] and have garnered considerable attention in the past decade as the synthetic modifications to their structure have become more accessible. A number of groups have shown that many different RNA molecules can bind aminoglycosides: 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 mRNA, and a variety of RNA aptamers from in vitro selection.[2] We have reported that aminoglycosides can stabilize DNA·RNA triplexes, hybrid duplexes, and that neomycin can even induce hybrid triplex formation.[3–6] While it stabilizes DNA triplex structures, neomycin does not affect DNA duplex stability (under physiological ionic conditions).[3]

In an effort to improve aminoglycoside–nucleic acid binding and develop more effective antibiotics, several novel neomycin conjugates have been reported, that include intercalator linked[4,7] as well as dimeric aminoglycosides linked via a long chain-alkyl linkage.[8,9] The dimeric aminoglycosides were shown to be more effective RNA binders than the individual aminoglycosides.[8] In our efforts to understand the remarkable ability of neomycin to stabilize triplex DNA, our work has shown the remarkable charge and shape complementarity of neomycin to the larger W–H groove. Several intercalator–neomycin conjugates have shown synergistic stabilization of the triplex structures as well.[4] The presence of additional positive charges and the size of dimeric conjugates (Scheme 1) prompted us to investigate their role in enhanced triplex groove recognition (perhaps through simultaneous major–minor groove interactions). We describe herein the unexpected nucleic acid stabilization results observed in the presence of a tobramycin–neomycin and neomycin–neomycin dimer (Scheme 1).

The poly(dA)·2poly(dT) triplex melt is seen at 34 °C and the duplex melts at 71 °C (150 mM KCl, pH 6.8 Fig. 1).[3] Upon increasing neomycin concentrations, the triplex melt increases without any effect on the duplex melt.[3,5] When a small amount of the neomycin–neomycin dimer was added to this triplex, the UV melts showed a remarkably surprising pattern (Fig. 1). The hypochromicity observed for the triplex melt simply decreases with a concomitant disappearance of the transition at a slightly higher dimer concentration ($r_{db} = 0.13$, where r_{db} is the ratio of the drug to the DNA). On the other hand, the duplex melt increases by 8 °C. To confirm that this behavior was not limited to the poly(dA)·poly(dT) structure, we looked at the smaller duplex $dT_{(16)}\cdot dA_{(16)}$. A Job plot of dA_{16} and dT_{16} shows the difference in selectivity for neomycin versus neomycin–tobramycin dimer at low temperature (Fig. 2). While the minimum is clearly seen at 66% dT_{16} in the presence of neomycin, neomycin–tobramycin dimer shows no such preference,

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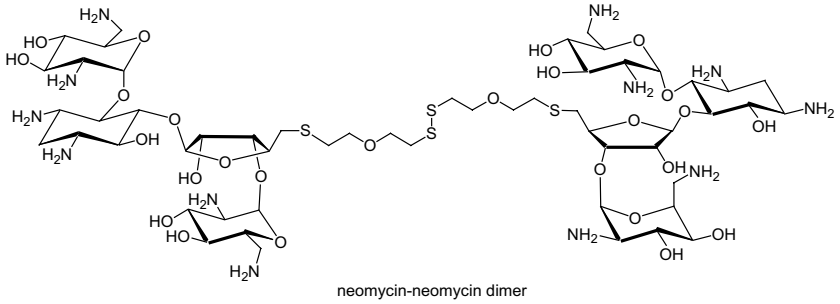
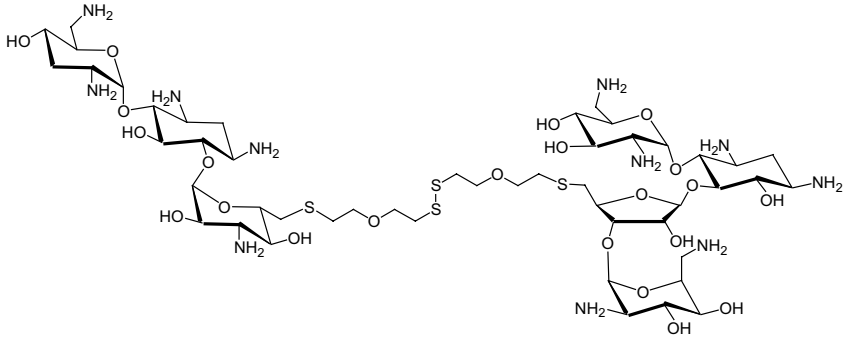


neomycin-neomycin dimer



neomycin-tobramycin dimer

Scheme 1. Structure of neomycin–neomycin and neomycin–tobramycin dimer used in the study.

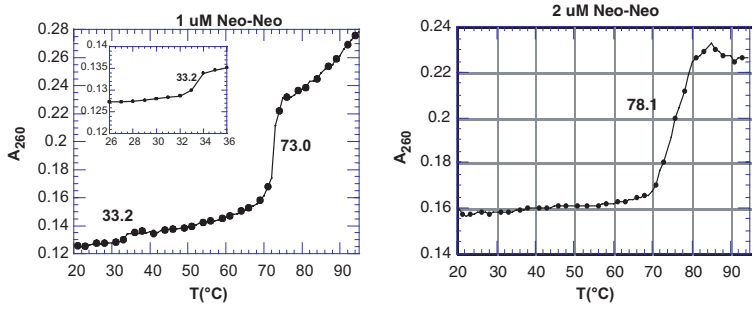


Figure 1. UV melting profiles of poly(dA)·2poly(dT) in the presence of 150 mM KCl at the indicated drug concentrations. [DNA] = 15 μM/base triplet. Solution conditions: 10 mM sodium cacodylate buffer, 0.5 mM EDTA, pH 7.2. Samples were heated from 20 to 95 °C at 5 deg/min, the annealing (95–20 °C) and the melting (20–95 °C) were conducted at 0.2 deg/min, and the samples were brought back to 20 °C at a rate of 5 deg/min.

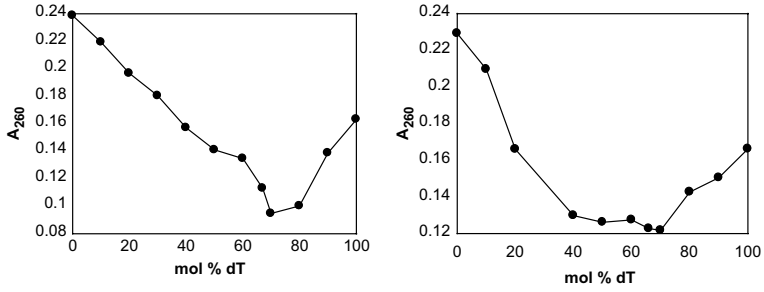


Figure 2. Job plot of dA_{16} (1.25 μM/strand) and dT_{16} (1.25 μM/strand) in the presence of added neomycin (left, $r_{db} = 0.66$) and neomycin–tobramycin dimer (right). Solution conditions: 10 mM sodium cacodylate, 0.5 mM EDTA, pH 6.8 at 10 °C.

but leads to a stabilization of the duplex such that a clear minimum between duplex/triplex is not distinguishable. Both dimers show similar UV thermal melt patterns at low concentrations (see supporting information).

Isothermal titration calorimetry was then carried out with neomycin–tobramycin dimer and poly(dA)·poly(dT) duplex (Fig. 3a,b; Table 1) as well as the poly(dA)·2poly(dT) triplex. A complete reversal of binding modes as seen for neomycin is seen with this dimer. Neo-

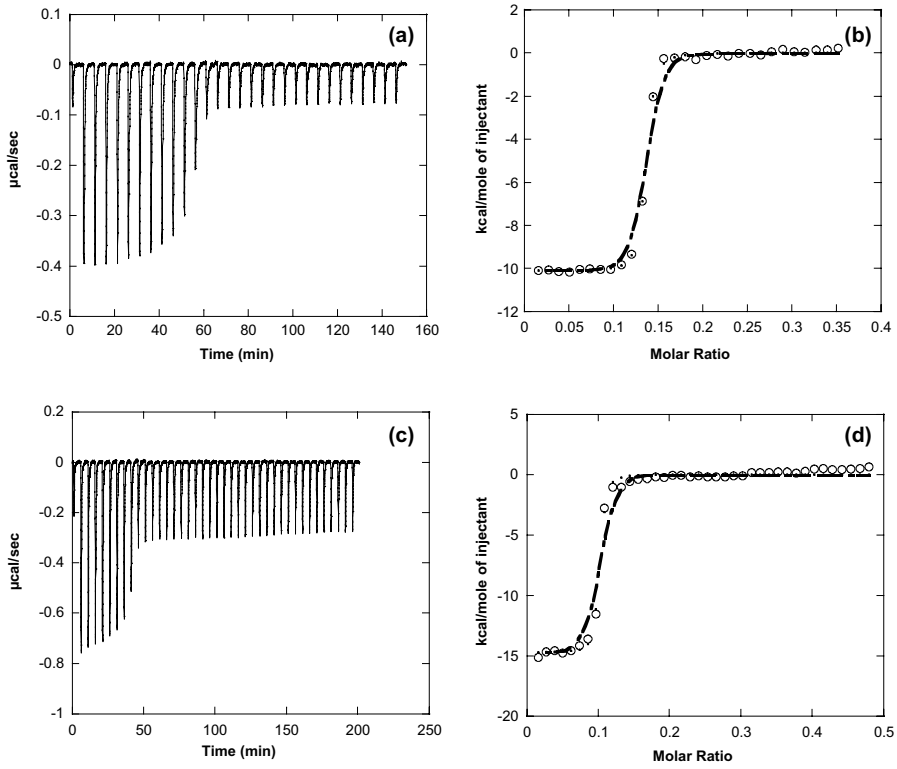


Figure 3. (a) ITC profile of poly(dA)·poly(dT) (60 µM/base pairs) titrated with neomycin–tobramycin conjugate (200 µM); (b) Corrected injection heats plotted as a function of the [drug]/[poly(dA)·poly(dT)] ratio; (c) ITC profile of poly(dA)·poly(dT) (60 µM/base pairs) titrated with neomycin–neomycin conjugate (200 µM); (d) Corrected injection heats plotted as a function of the [drug]/[poly(dA)·poly(dT)] ratio. Condition: 10 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl, pH 6.8 at 20 °C.

Table 1. ITC-derived thermodynamic profiles for the binding of neomycin conjugates to poly(dA)·poly(dT) double helix in 10 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl, pH 6.8 at 20 °C

Drug	N bp/drug	K_a ($10^8\,M^{-1}$)	ΔH (kcal mol^{-1})	$T\Delta S$ (kcal mol^{-1})	ΔG (kcal mol^{-1})
Neomycin–tobramycin	7.64 ± 0.015	1.1 ± 0.1	−10.12 ± 0.036	0.67	−10.79
Neomycin–neomycin	10.31 ± 0.05	1.15 ± 0.26	−14.73 ± 0.14	−3.9	−10.83

mycin shows nonspecific electrostatic binding with the DNA duplex and a single high affinity binding site with the DNA triplex; neomycin–tobramycin dimer however, shows a high affinity binding site with the DNA duplex, mainly driven by a large negative enthalpy. While neomycin gives a high association constant with the poly(dA)·2(dT) triplex (4.5 base triplets/drug binding site),[10] neomycin–tobramycin dimer leads to a K_a of $1.0\times10^8\,M^{-1}$ in binding to the poly(dA)·(dT) duplex (7 base pair/drug). Multiple binding sites were observed in titration of the dimer to the poly(dA)·2poly(dT) triplex, which could not be fit to available models (shown in supporting information). Additionally, titration of neomycin–neomycin dimer (Fig. 3c,d, Table 1) to the poly(dA)·(dT) duplex yields an association constant similar to that observed with the neomycin–tobramycin dimer (with an even higher enthalpy contribution to binding). This suggests that the shape/charge complementarity to the duplex groove is perhaps more important than specific atom contacts made by any ligand.

What then is the cause of this surprising duplex stabilization by these dimers: The major groove of the duplex remains a plausible binding site, and is substantiated by the following observations: (1) triplex destabilization at low drug concentrations (blocking the third strand from the major groove. (2) No stabilization of the DNA duplex by neomycin or tobramycin (indicating that these ligands do not occupy the DNA minor groove).[3] (3) A larger binding site (7–10 base pairs) and a good charge/shape complementarity of the dimer to the DNA major groove. The dimeric conjugate can take a conformation mimicking the triplex third strand such that the two ends of the groove are held together by H-bonds/electrostatic complementarity (neomycin alone is unable to do that and has been shown to have a better charge/shape complementarity to the triplex W–H groove or other A-form major grooves).[10]

Few carbohydrate ligands have ever been known to show such high-affinity binding to duplex structures.[11] Major groove DNA binding ligands (unlike minor groove binders) are limited to protein structures and a few small ligands. We have previously reported that neomycin (and other aminoglycosides) can stabilize DNA/RNA triplexes, hybrid duplexes, hybrid triplex

and even stabilize tetraplexes.[3,6,7,10,12] While it stabilizes DNA triplex structures, neomycin does not affect DNA duplex stability (under physiological ionic conditions).[3] Our previous work has also suggested that aminoglycoside specificity (neomycin in high nM–low μM range) may be for nucleic acid forms that show some features characteristic of an A-type conformation {RNA triplex, DNA–RNA hybrid duplex, RNA duplex, DNA triplex, A-form DNA duplex, and DNA tetraplex}, rather than for naturally occurring RNA.[12] Neomycin fits better in the narrower A-form major groove but does not have a good charge or shape complementarity to the major groove of B-form DNA. We have previously shown that a Hoechst–neomycin conjugate can force neomycin into the larger B-form DNA duplex groove.[13] In retrospect, the larger size of the B-form major groove could be a surprisingly good fit for dimeric aminoglycosides.[8] Groove recognition of A and B-form duplexes and triplexes, and even higher order structures can then be made possible if one carefully applies the principles of charge/shape complementarity to nucleic acid recognition. Aminoglycosides, with their unique positively charged manifold present us with such a new motif of recognition for these higher order RNA/DNA nucleic acid forms. The results of our experiments described here identify a new duplex groove binding ligand selective for B-form DNA duplexes. A detailed examination of the sequence-specificity, mode of binding, and duplex versus triplex selectivity of these dimeric structures is now being investigated and will be reported in the near future.

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Supplementary material

Experimental conditions and ITC profiles for neo-tob binding to poly(dA)·poly(dT) triplex can be found, in the online version, at doi:10.1016/j.bmcl.2004.07.002.

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