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DNA Triple Helix Stabilization by Aminoglycoside Antibiotics

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Abstract—The stabilization of the poly(dA)•2poly(dT) triple helix by neomycin is reported. Preliminary results indicate that neomycin stabilizes DNA triple helices and the double helical structures composed of poly(dA)•poly(dT) are virtually unaffected. This is the first report of the interaction of aminoglycoside antibiotics with DNA triple helices.

Introduction

Association of a DNA third strand with a duplex is a thermo-dynamically weaker and a kinetically slower interaction than duplex formation itself.[1,2] Rates of triple helix formation ($10–10^3$ M^{-1} s^{-1}) [1,3,4] are slow (3–4 orders of magnitude) compared to the rate of duplex recombination ($\sim 10^6$ M^{-1} s^{-1}).[2,5] Most ligands that stabilize triple helices either intercalate, bind in the minor groove and/or carry positively charged functional groups.[6] The intercalating ligands (fused-ring polycyclic compounds, benzo [*e*]pyridoindole derivatives,[7] ethidium,[8,9] the alkaloid coralyne[10]) generally tend to be nonspecific in the triplex to duplex stabilization, although advances have been made to improve that ratio.[7,11] Established DNA minor groove-binding ligands, including berenil,[12] 4′-6-diamidino-2-phenylindole,[9] netropsin,[9] are also effective stabilizers for nucleic acid triplexes when at least one strand is a ribooligonucleotide. Recently, 3,3′-diethyl-oxadicarbocyanine has been shown to selectively stablilize DNA triple helical structures.[13] Polycations like diamines and polyamines have been known to stabilize triple helical structures for a long time.[14–16] In our quest for new ligands for triple helix stabilization, we have investigated aminoglycoside antibiotics (Scheme 1) for stabilization of triple helical nucleic acid structures.

Aminoglycoside antibiotics are bactericidal agents that are comprised of two or more amino sugars joined in glycosidic linkage to a hexose nucleus. Recent work by Rando,[17–19] Tor,[20,21] and other groups[22] have shown that the binding of aminoglycosides is favored at domains in RNA that are nonduplex in nature.[18] Wong has recently shown that 1-amino-3-propanols can bind to phosphodiesters with better affinity than guanidinium groups.[23] Recent studies have also found many RNA molecules that can bind aminoglycosides: group I introns, a hammerhead ribozyme,[21] the RRE transcriptional activator region from HIV,[24,25] which contains the binding site for the Rev protein, and a variety of RNA aptamers from in vitro selection. The asymmetric structure of the aminohexoses in these antibiotics prompted us to investigate if these potentially non-intercalative compounds would preferentially stabilize a triple helix over a duplex structure. We report, herein, the stabilization of the poly(dA)•2poly(dT) triple helix by neomycin. Preliminary results indicate that neomycin stabilizes DNA triple helices and the double helical structures composed of poly(dA)•poly(dT) are virtually unaffected.

Results and Discussion

In the thermal denaturation analysis of poly(dA)•2-poly(dT) bound to neomycin (Table 1, for melting curves see supporting information), plots of absorbance at 260 nm (A_{260}) versus temperature exhibit two distinct inflections ($T_{m1} = 34\,^{\circ}C$ and $T_{m2} = 71\,^{\circ}C$, $\mu = 0.15$).[13] During the first transition, annealing and melting curves of complexes formed from poly(dA)•2poly(dT) with neomycin exhibit hysteresis at the rate of heating-cooling employed (0.5 and 0.2 deg/min) (see supporting information). Thermal analysis at 284 nm only show hyperchromicity for the transition of the triple helix to the double helix. These measurements confirm that the first transition (T_{m1}) is the destabilization of the triple helix, since: (a) rates of formation of triple helices are

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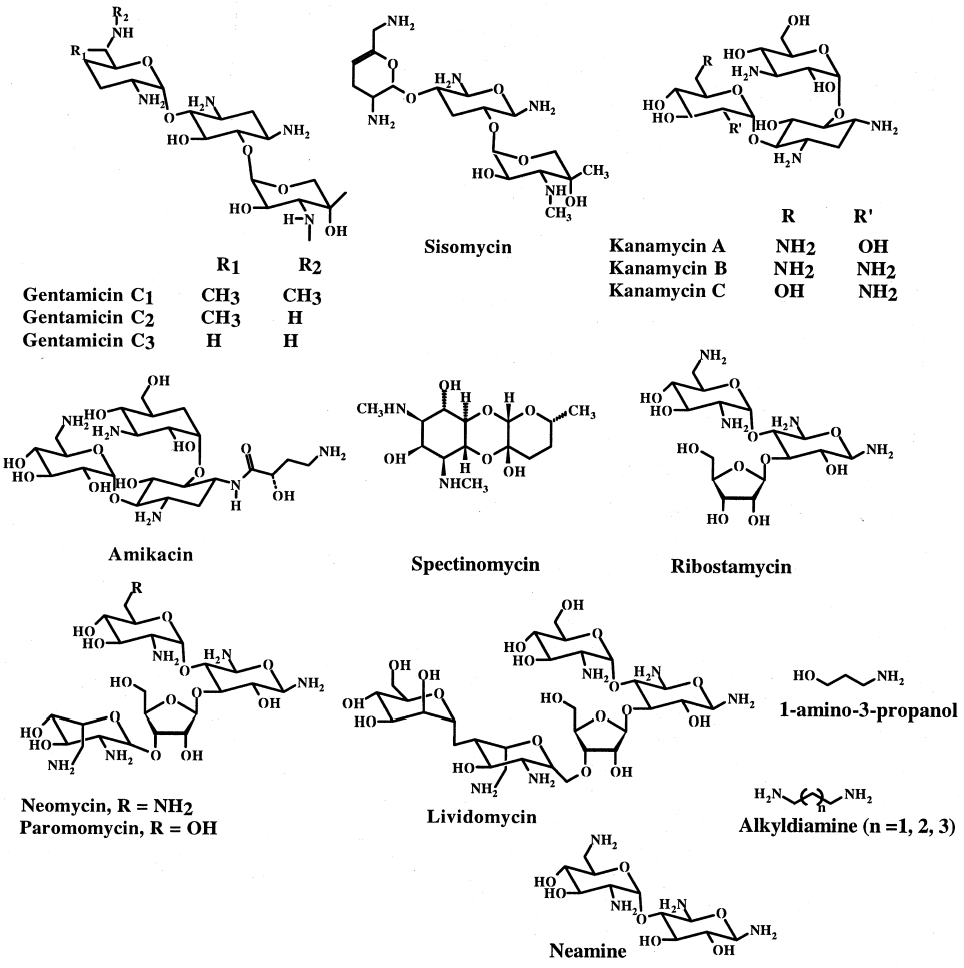

Scheme 1. Structures of aminoglycosides/amines used in the study.

considerably lower than double helical complexes[1] and (b) triple helical transitions show hyperchromic effect at ~280 nm.[5] Increasing the concentration of neomycin stabilizes the triple helical structure as evidenced by thermal denaturation studies (Table 1). Table 1 shows that by increasing the molar ratios of neomycin from 0–25 μM, r_{bt} (ratio-base triplet/aminoglycoside) = 15–0.6, the triplex melting point is increased by close to 25 °C, whereas the duplex is virtually unaffected. It is remarkable that under these conditions, neomycin has little or no effect on the duplex DNA melting. This was independently confirmed by using double helical poly(dA)·poly(dT). In the absence of KCl, only one transition is seen, which corresponds to the melting of the duplex. Addition of 4 μM neomycin in the absence of any salt leads to two clear transitions, driving the equilibrium in eq (1) to the right (see supporting information).

Table 1. UV melting temperatures at 260 nm at indicated neomycin concentrations in the presence of 150 mM KCl

Poly(dA)·2Poly(dT) + Neomycin (μM)	T_{m1}	ΔT_{m1}	T_{m2}	ΔT_{m2}
0	34.0	0.0	71.0	0.0
1	36.1	2.1	72.0	1.0
2	36.2	2.2	71.0	0.0
4	39.7	5.7	72.0	1.0
10	49.5	15.5	71.0	0.0
15	54.5	20.5	71.0	0.0
20	56.3	22.3	73.0	2.0
25	58.7	24.7	72.0	1.0

$$dA\cdot dT + dT \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} dA\cdot 2dT \quad (1)$$

Since neomycin does not interact with duplex DNA under the conditions of this assay, its triplex selectivity is more pronounced than of other triplex binders as BePI,[7] coralyne,[10] and quite similar to the selective stabilization recently shown by 3,3′-diethyloxadicarbocyanine.[13] What then is the cause of triple helical stabilization by neomycin? Is it simply the electrostatic effect of the positively charged amines? We carried out thermal analysis of poly(dA)·2poly(dT) in the presence of equimolar diamines, aminopropanol, neamine and other aminoglycosides (Scheme 1). Table 2 shows the results of the thermal analysis of poly(dA)·2(dT) in the presence of aminoglycosides. Most aminoglycosides (4–10 μM, r_{bt} = 3.75–1.5) have either no effect or slightly destabilize the triple helix. Sisomycin, paromo-mycin, and lividomycin are the only other antibiotics that have a stabilizing effect. The difference between the effectiveness of paromomycin and neomycin is quite remarkable. The

Table 2. UV melting temperatures at 260 nm at the indicated aminoglycoside concentration in the presence of 150 mM KCl

Poly(dA)·2Poly(dT) + Aminoglycoside Antibiotics	4 μM			10 μM		
	T_{m1}	ΔT_{m1}	T_{m2}	T_{m1}	ΔT_{m1}	T_{m2}
Neomycin	39.7	5.7	72.0	48.6	14.6	71.0
Neamine	34.0	0.0	71.0	34.1	0.1	71.1
Lividomycin	36.1	2.1	72.1	36.3	2.3	72.1
Amikacin	34.6	0.6	71.0	35.2	1.2	71.0
Spectinomycin	33.0	−1.0	71.0	34.0	0.0	71.0
Ribostamycin	31.7	−2.3	71.0	33.4	−0.6	71.1
Sisomicin	36.1	2.1	71.0	38.6	4.6	71.1
Kanamycin	31.7	−2.3	71.0	33.4	−0.6	71.1
Gentamycin	34.7	0.7	71.0	37.3	3.3	71.1
Paromomycin	36.2	2.2	71.1	39.3	5.3	72.0

structural difference between the two is a positively charged amino group (present in neomycin), replacing a neutral hydroxyl (present in paromomycin). This leads to a difference of 10 °C in T_{m1} values ($r_{bt} = 1.5$). At lower concentrations of antibiotics ($r_{bt} = 3.75$), paromomycin has little effect on the stability of the triplex. Lividomycin, a paromomycin analogue with a polyhydroxy hexose tether, is less effective than paromo-mycin in increasing T_{m1} values under these conditions. While neamine (structural subset of neomycin—Scheme 1, 4 positive charges), the diamines and aminopropanol (4–10 μM amine) have little or no effect on the stabilization of the duplex or the triplex, simple diamines and aminopropanol actually destabilize the triple helix as well as the double helix when their concentrations are increased (4 mM, see supporting information). This is not unprecedented[16] since simple point charges that do not bind appreciably to the helix would compete for the salt ions in the solution and hence destabilize the triple helix. This suggests that the polycationic nature of neomycin is not the only explanation for its role in triple-helical stabilization. The rigid conformation of this constrained aminoglycoside makes it more suitable for triplex binding than the flexible conformation of some di and polyamines. Geometrical parameters, for example, the distance between the charges must also play a significant role.[15,26] Since neomycin's structure prohibits intercalation, it must be binding to one of the triplex grooves. Based on this assumption, neomycin is the most selective triplex groove binder described to date.[12,13]

To the best of our knowledge, this is the first example of the interaction of aminoglycoside antibiotics with triple-stranded nucleic acid structures. Neomycin is shown to be the most effective in stabilizing poly(dA)·2poly(dT) triplex and selectively raises the stability of the triplex over duplex. Our findings suggest that these antibiotics may be able to aid H-DNA formation in vivo and could have an alternative mode of action that has been previously unexplored. Further studies aimed at better understanding the mode of triplex binding and the role of these antibiotics in mixed sequences/H-DNA formation are being carried out in our laboratories and should lead to the development of novel triple helix stabilizing agents.

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Supporting Information Available

Melting and annealing curves of {poly(dA)·2poly(dT)} at 260, 284 nm, tables of melting points in the presence of neomycin and other amines (11 pages).

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