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Research paper

Calorimetric and spectroscopic studies of aminoglycoside binding to AT-rich DNA triple helices

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ABSTRACT

Calorimetric and fluorescence techniques were used to characterize the binding of aminoglycosides-neomycin, paromomycin, and ribostamycin, with 5′-dA_{12}-x-dT_{12}-x-dT_{12}-3′ intramolecular DNA triplex (x = hexaethylene glycol) and poly(dA)·2poly(dT) triplex. Our results demonstrate the following features: (1) UV thermal analysis reveals that the T_m for triplex decreases with increasing pH value in the presence of neomycin, while the T_m for the duplex remains unchanged. (2) The binding affinity of neomycin decreases with increased pH, although there is an increase in observed binding enthalpy. (3) ITC studies conducted in two buffers (sodium cacodylate and MOPS) yield the number of protonated drug amino groups (Δn) as 0.29 and 0.40 for neomycin and paromomycin interaction with 5′-dA_{12}-x-dT_{12}-x-dT_{12}-3′, respectively. (4) The specific heat capacity change (ΔC_p) determined by ITC studies is negative, with more negative values at lower salt concentrations. From 100 mM to 250 mM KCl, the ΔC_p ranges from −402 to −60 cal/(mol K) for neomycin. At pH 5.5, a more positive ΔC_p is observed, with a value of −98 cal/(mol K) at 100 mM KCl. ΔC_p is not significantly affected by ionic strength. (5) Salt dependence studies reveal that there are at least three amino groups of neomycin participating in the electrostatic interactions with the triplex. (6) FID studies using thiazole orange were used to derive the AC_{50} (aminoglycoside concentration needed to displace 50% of the dye from the triplex) values. Neomycin shows a seven fold higher affinity than paromomycin and eleven fold higher affinity than ribostamycin at pH 6.8. (7) Modeling studies, consistent with UV and ITC results, show the importance of an additional positive charge in triplex recognition by neomycin. The modeling and thermodynamic studies indicate that neomycin binding to the DNA triplex depends upon significant contributions from charge as well as shape complementarity of the drug to the DNA triplex Watson–Hoogsteen groove.

Considerable attention has been given to oligonucleotide-directed triple helix formation of nucleic acids [1–3] due to its possible regulatory role *in vivo* [4] and its potential applications in medicine and biotechnology [5,6]. In the intermolecular triplex system, the double helix associates with a single-strand triple helix-forming oligonucleotide (TFO) via Hoogsteen hydrogen bonds in the major groove. Because TFOs bind in the major groove of duplex DNA, they can be used as transcription inhibition agents [7]. TFOs have been used to study the molecular mechanism of triplex-mediated TCR (transcription-coupled repair) in HeLa nuclear extracts, and the formation of a triplex induced much stronger DNA repair activity in promoter-containing plasmids [8]. A 17-mer homopyrimidine oligonucleotide has been shown to bind to the major groove of SV40 DNA, inhibiting enzymatic cleavage [9]. Catapano has shown the suitability of a triplex based approach using phosphorothioate-linked oligos in targeting the C-myc and Ets-2 transcription factors [10–12]. A number of reviews on the potential of DNA triplex in DNA targeted diagnostics and drugs have appeared in the recent literature [13–18].

The triplex structure contains two motifs: purine and pyrimidine (Scheme 1). In the purine motif, the homopurine third strand binds antiparallel to the purine strand of the duplex by reverse-Hoogsteen hydrogen bonds [19–21]. In the pyrimidine motif, the third strand composed of pyrimidine bases binds parallel to the purine strand of the Watson–Crick duplex by forming Hoogsteen hydrogen bonds. However, in the intramolecular triplex, first found to exist in biological systems in 1986 [22], triplex formation is different from that of intermolecular triplex. In this case, the homopurine–homopyrimidine mirror repeats within the DNA

Abbreviations: ITC, Isothermal titration calorimetry; DSC, Differential scanning calorimetry; MOPS, 3-[*N*-morpholino] propanesulfonic acid; HS, Hoogsteen; WC, Watson–Crick; W–H, Watson–Hoogsteen; FID, Fluorescence intercalator displacement.

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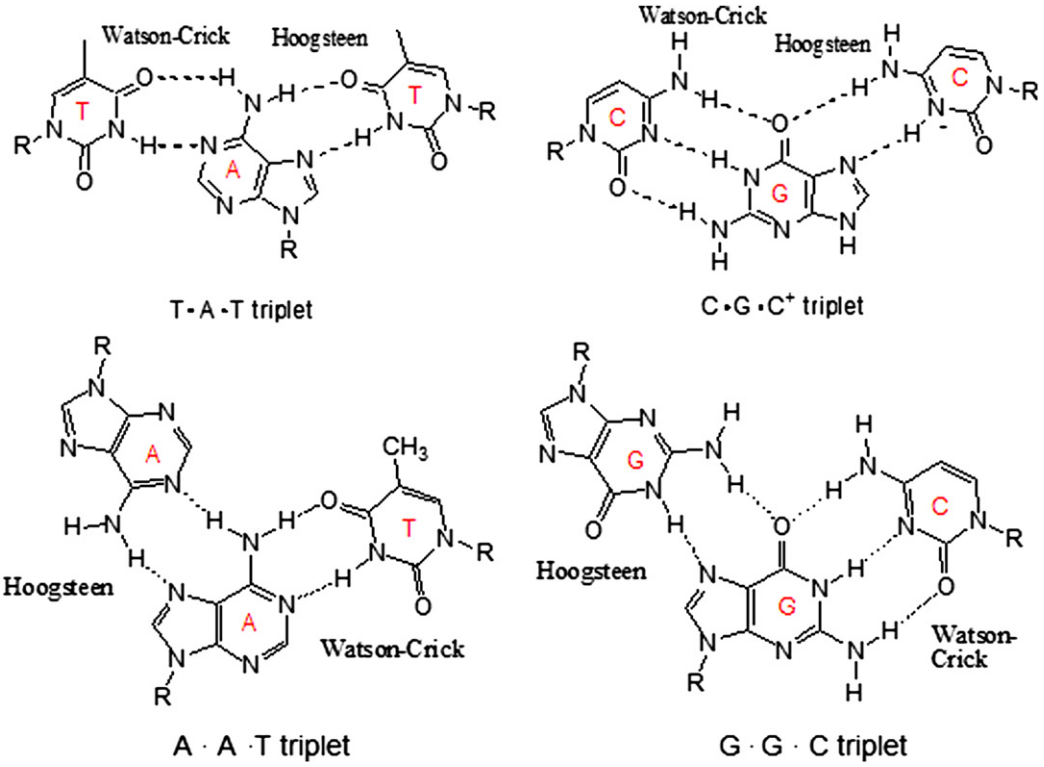

Scheme 1. Hydrogen bonds formed in pyrimidine·purine-pyrimidine triplets (pyrimidine motif) and purine·purine-pyrimidine triplets (purine motif).

duplex denature to allow one strand to fold back, forming Hoogsteen hydrogen bonds with the adjacent DNA duplex sequence. The intramolecular triplex, known also as H-DNA [21], is favored under negative DNA supercoiling conditions [23], acidic solutions, or in the presence of divalent cations [24]. The intramolecular triplex has also been reported to have an effect on the transcriptional regulation and replication *in vivo* [25–27]. H-DNA, found in *Escherichia coli* and eukaryotic cells [28–30], is found in promoter regions and around recombination hot spots [31]. Therefore, it likely plays a regulatory role in gene expression [32], recombination [33], and in transcription of human viruses [34]. It has also been implicated in the suppression of the human γ-globin gene [35].

However, the DNA triplex is not as stable as its corresponding duplex. The association of a third strand with a duplex ($k_{on} \sim 10 - 10^3\ M^{-1}\ s^{-1}$) is a much slower process than the association of two single strands in forming a duplex ($k_{on} \sim 10^6\ M^{-1}\ s^{-1}$) [36–38]. Many studies have been carried out using small molecules to improve the thermal stability of DNA triple helices [39–49]. However, these ligands do not selectively bind to the DNA triple helices, and some even destabilize triple helices [38]. Our previous work has shown the remarkable ability of neomycin, its conjugates with other intercalators and other aminoglycosides (Scheme 2), to stabilize DNA, RNA and hybrid triple helices [38,39,50–53]and to even aid the delivery of PS-modified TFOs into cancer cells [54]. In particular, neomycin was found to induce the stabilization of a DNA.DNA.DNA [50] triple helix, a DNA.RNA hybrid duplex [39,55], as well as DNA.DNA.RNA. hybrid triple helices [39], significantly adding to the number of nucleic acids (other than RNA) that aminoglycosides have been shown to target [56]. Among the aminoglycosides studied, it was found that neomycin significantly stabilized DNA and RNA triple helices [38]. Herein, we report our observations regarding the role of aminoglycosides in the thermal stabilization of AT-rich triplexes and their interactions with aminoglycosides from a thermodynamic perspective.

2. Methods and materials

2.1. Nucleic acids and aminoglycosides

The intramolecular triplex (5′-dA_{12}-x-dT_{12}-x-dT_{12}-3′) was synthesized using an Expedite Nucleic Acid Synthesis System (8909) with standard phosphoramidite chemistry. The oligomer was purified on an anion exchange HPLC column (Water Gen-Pak FAX, 4.6 × 100 mm) with a Tris·HCl buffer system. Buffer A: 25 mM Tris·HCl, 1 mM EDTA, and 10% MeCN (v/v%); Buffer B: Buffer A + 1 M NaCl. Conditions: 2–60% buffer B over buffer A during 0–16 min at a flow rate of 0.75 mL/min. Neomycin sulfate and ribostamycin were obtained from ICN Biomedicals Inc., and paromomycin sulfate was purchased from Sigma. Neomycin sulfate was protected with Boc anhydride, chromatographed on silica gel and deprotected to obtain pure neomycin B. All aminoglycosides were used without further purification. All the polynucleotides were purchased from GE Healthcare Amersham Bioscience. The concentrations of polymer solutions were determined spectrophotometrically using the following extinction coefficients (in units of mol of nucleotide or $bp/L^{-1}\ cm^{-1}$): $\varepsilon_{264} = 8520$ for poly(dT), $\varepsilon_{260} = 6000$ for poly(dA)·poly(dT); 5′-dA_{12}-x-dT_{12}-x-dT_{12}-3′, $\varepsilon_{260} = 341{,}100$.

2.2. UV spectrophotometry

All UV absorbance experiments were conducted on a Cary 1E UV/vis spectrophotometer equipped with temperature programming. Quartz cells with a 1 cm path length were used for all the absorbance studies. For the triplex preparation, all samples were heated at 95 °C for 5 min, then cooled slowly to room temperature and allowed to incubate for 16 h at 4 °C prior to use. Absorbance vs. temperature profiles were recorded at 260 nm and 280 nm. The samples were heated from 5 °C to 95 °C at a rate of 0.2 °C/min, then