Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 615–618

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Absorption Studies on Aminoglycoside Binding to the Packaging Region of Human Immunodeficiency Virus Type-1

Julie M. Sullivan, Jerry Goodisman and James C. Dabrowiak*

Department of Chemistry, CST 1-014, Syracuse University, Syracuse, NY 13244, USA

Received 16 October 2001; accepted 28 November 2001

Abstract—The binding of paromomycin, neomycin B, and three analogues of neomycin to a 176-mer RNA from the packaging region of HIV-1 (LAI) has been studied using UV absorption spectroscopy at wavelengths between 200 and 300 nm. From plots of absorption as a function of drug concentration, values of binding constants for these drugs on RNA were determined. © 2002 Elsevier Science Ltd. All rights reserved.

Aminogylcoside drugs are cationic natural products that interact with RNA.[1] Their bactericidal effects stem from their ability to block protein synthesis by binding to the A-site on ribosomal RNA.[1–3] This, and the fact that their structures can be chemically modified, suggest that aminoglycoside analogues may be useful for treating certain diseases. For example, the genetic information in human immunodeficiency virus and various tumor viruses is in the form of RNA.[4] Since the genomes of these viruses likely have unique structures, it may be possible to design agents that selectively block virus proliferation by targeting a specific site on RNA.[5–12]

In this report, we use UV absorption spectroscopy to monitor aminoglycoside binding to a 176-mer RNA from the packaging region of HIV-1 (LAI) (Fig. 1). This segment of RNA, critically important in a number of steps in the life cycle of the virus,[4] is a potential target for drug action. In a previous report,[13] we used quantitative footprinting methods to identify paromomycin binding sites on the 176-mer (Fig. 1), and find regions that undergo structural change when the drug binds. In this work, we examine the effects of paromomycin, neomycin B, and three neomycin analogues: guanidino-neomycin,[14] neo-acridine,[15] and neo-neo[16] (Fig. 1) on the absorption spectrum of this RNA.

The 176-mer RNA was synthesized using T7 RNA polymerase and purified by PAGE. Its extinction coefficient was determined by hydrolysis with base.[13] The secondary structure shown in Figure 1, determined using the program Mfold,[17] shows the four stem-loops found in HIV-1 (LAI), SL 1–4,[18] and a main stem produced by the HIV-1 sequences flanking SL 1–4. The 176-mer can form a dimer, but gel electrophoresis showed that, in the low ionic strength buffer (10 mM Tris, pH, 7.0) and at the low RNA concentration used here (0.53 μM), it is monomeric. Drug concentrations were varied by adding a measured amount of stock solution of the drug to RNA (~20 °C) and allowing the system to come to equilibrium (within the time of mixing). UV absorption was measured in the range 200–300 nm. After correction for dilution (less than 10%), plots of absorbance versus drug concentration at various wavelengths were constructed. Discontinuities in some of these plots (for all but paromomycin, these occurred at 10–15 μM drug) correlated with drug-induced precipitation of RNA. This was checked by measuring absorbance before and after centrifugation (Minifuge) of selected drug–RNA solutions.

Figure 2 shows, first, absorption spectra of the 176-mer in the presence of several concentrations of paromomycin, and, following, plots of absorbance versus drug concentration at several wavelengths for each of five compounds. Data are for concentrations below that producing precipitation. Each set of data was analyzed according to a two-state model; that is it was assumed that free RNA, free drug, and RNA with drug bound were in equilibrium according to $K = [RD]/\{[R][D]\}$, where $[D] + [RD] =$ total drug concentration D_t and $[R] + [RD] =$ total RNA concentration R_t. At a given

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*Corresponding author. Tel.: +1-315-443-4601; fax: +1-315-443-4070; e-mail: jcdabrow@syr.edu

0960-894X/02/$ - see front matter
PII: S0960-894X(01)00823-X

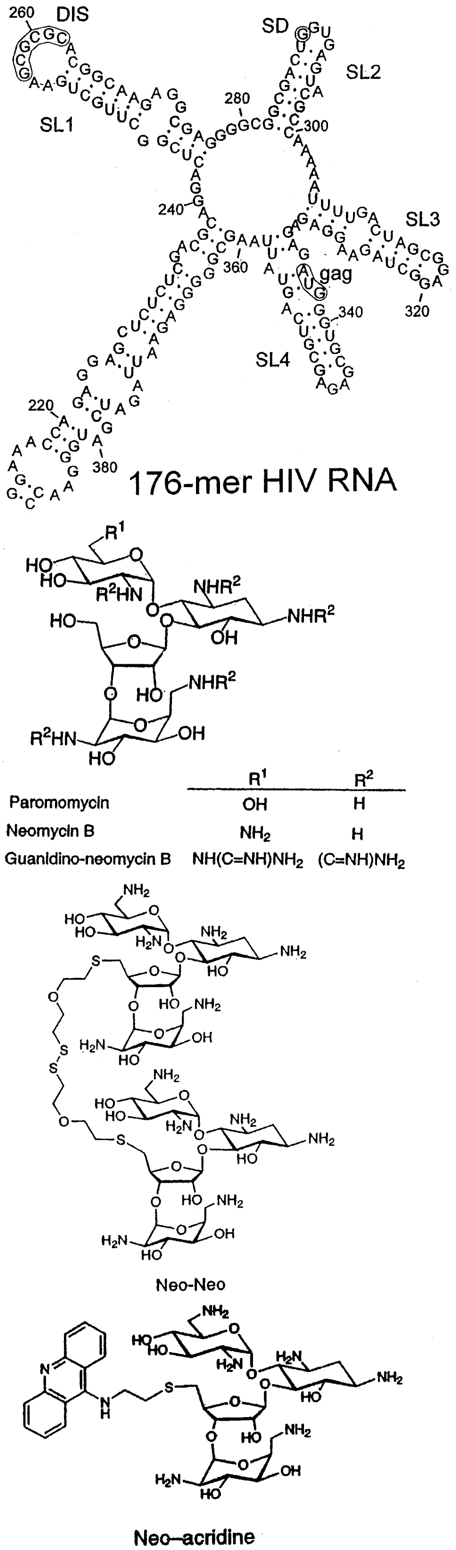

Figure 1. Sequence and secondary structure of the 176-mer from the packaging region of HIV-1 (LAI) is shown. The RNA consists of the main stem, sites 213–238 and 361–388; SL 1, which contains the Dimer Initiation Site (DIS); SL 2, having the 5′ splice donor site (SD); SL 3; and SL 4, the last containing the start codon for the *gag* gene. The structures of the aminoglycosides used in the study are also shown.

wavelength, the absorbance was calculated as $[RD]/R_t$ times the absorbance of drug-bound RNA + $[R]/R_t$ times the absorbance of free RNA. The value of K was determined to give the best fit between calculated and measured absorbance for from 8 to 15 wavelengths (same K used for all). The smooth curves in Figure 2 represent calculated absorbances for each wavelength.

For the drugs paromomycin, neomycin, neo-acridine, neo-neo, and guanidino-neomycin, we find $K = (4.3 \pm 1.9) \times 10^4$, 1.2×10^5, 1.6×10^4, 3.8×10^3, and 1.0×10^5 M^{-1}. (The stated error in K for paromomycin is the variation in K required to increase the sum of the squared deviations of theoretical from experimental absorptions by 10%.) We assume only a single binding event is involved. However, the low-concentration behavior of the plots for paromomycin, neo-neo, and guanidino-neomycin (see Fig. 2) indicates the existence of binding events with a high K value. For these drugs, we analyzed the low-concentration points for all the wavelengths according to a two-state model and determined K values of $(1.8 \pm 1.2) \times 10^6$, 1.5×10^7, and 5.5×10^7 M^{-1}, respectively. (Uncertainties are large because so few data points were used.) Analysis of circular dichroism as a function of paromomycin concentration (data not shown) yields a K of several times 10^6 M^{-1}, so absorption and CD may be detecting the same binding event. Quantitative analysis of footprinting data (not shown) did not reveal any K this large. However, there is considerable scatter in the data and, due to limited cutting by the probe (RNase I), a significant part of the 176-mer was not accessible to footprinting measurements.

Since a decrease in the amount of base stacking is expected to cause an increase in absorbance at 260 nm, neo-neo, neo-acridine and guanidino-neomycin *reduce* base stacking (Fig. 2). In contrast, paromomycin and neomycin cause absorbance decreases, indicating that they *increase* base stacking.[19] Interestingly, neomycin, which is structurally similar to paromomycin, does not exhibit a detectable binding event at low drug concentration (Fig. 2). Since the only difference between paromomycin and neomycin is the group on C-5′ of ring I of the structure, this group appears to be important in determining the amount of base stacking in the high-affinity binding site.

In principle, the neo-neo analogue can bind using both neomycin units (bivalent binding), but the value of K for this analogue is similar to that of the other compounds. Thus, neo-neo appears to bind through only one of its neomycin units (monovalent binding), suggesting that the 176-mer lacks two close-together high affinity neomycin sites. After accounting for differences in ionic strength, the value of K measured in this study for neo-neo is comparable to that found for monovalent binding of the same analogue to A-site RNA ($I \sim 168$ mM; K, 3×10^6 M^{-1}).[20]

The compound guanidino-neomycin has been previously shown to be about 100 times more potent at inhibiting replication of HIV in Hela cells than neomycin.[14] The basis for the anti-HIV activity of guanidino-neomycin is

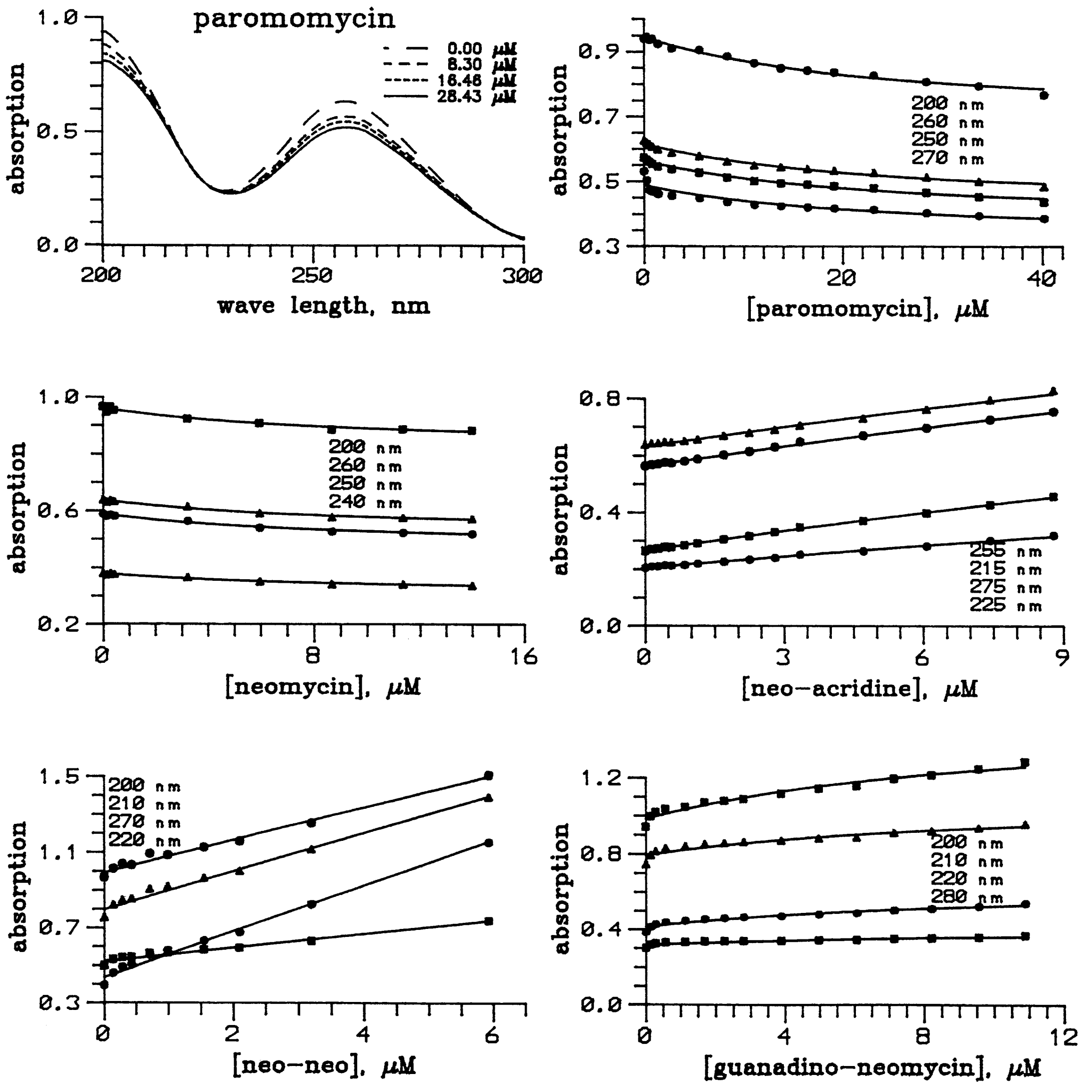

Figure 2. Absorption spectra of the 176-mer in the presence of various amounts of paromomycin is shown in the upper left panel. The other plots show absorbance as a function of concentration at selected wavelengths for the various drugs. Absorbance calculated using the two-state model with best-fit parameters (see text) are shown as smooth curves. The wave lengths and concentrations given correspond to the curves shown (top to bottom).

not known, but this compound has the highest binding constant toward the 176-mer. Further work will be necessary to determine to what extent guanidino-neomycin can inhibit dimer formation protein binding associated with the 176-mer.

We have used absorption spectroscopy to study aminoglycoside binding to a 176-mer RNA from the packaging region of HIV. Fitting the absorption changes to a two-state model, we derived binding constants for paromomycin, neomycin and three neomycin analogues. The size of the binding constants for the high-affinity sites is comparable to those found for other RNAs using other techniques.[20–22]

Acknowledgements

We thank NSF-REU (CHE 9987838) for financial support to J.M.S. We are grateful to M. P. McPike, for technical assistance and samples of the 176-mer RNA and we also wish to thank Y. Tor (U. C. San Diego) for the neomycin analogues and R. H. Shafer (U. C. San Francisco) for helpful discussions. Special thanks to Andrew Paoletti for sharing the results of electrophoresis experiments on a 154-mer RNA.

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19. The ability of neomycin to perturb the absorption spectrum of the four ribonucleotide triphosphates, which are devoid of secondary structure, was measured. For three of these, there was no measurable effect of drug on absorption for values of r = [drug]/[nucl] from 0 to x, where $x = 0.4$ (rATP), 0.15 (rCTP), and 0.22 (rGTP). The decrease in ε_{260} for UTP at r = 0.23 was ~8%. Thus, absorbance changes for the 176-mer in the presence of the drugs are mostly due to changes in base stacking.
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