Aminoglycoside Binding to the Hammerhead Ribozyme: A General Model for the Interaction of Cationic Antibiotics with RNA

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A variety of drugs inhibit biological key processes by binding to a specific RNA component. We focus here on the well-analysed hammerhead ribozyme RNA that is inhibited by aminoglycoside antibiotics, a process considered as a paradigm for studying drug/RNA interactions. With insight gained from molecular dynamics simulations of the ribozyme in the presence of Mg$^{2+}$ identified by crystallography and of aminoglycosides in solution, a general model for aminoglycoside binding to RNA is proposed. A striking structurally based complementarity between the charged ammonium groups of the aminoglycosides and the metal binding sites in the hammerhead was uncovered. Despite dynamical flexibility of the aminoglycosides, several of the intramolecular distances between the charged ammonium groups of the drugs were found to be rather constant. Intramolecular ammonium distances of the aminoglycosides span ranges similar to the interionic distances between Mg$^{2+}$ in the hammerhead. Successful docking of aminoglycosides to the hammerhead ribozyme could be achieved by positioning the ammonium groups at the sites occupied by Mg$^{2+}$. The covalently linked ammonium groups of the aminoglycosides are thus able to complement in space the negative electrostatic potential created by a three-dimensional RNA fold. Consequently, it is suggested that aminoglycoside-derived sugars could constitute a basic set of yardstick synthons ideal for rational and combinatorial synthesis of drugs targeted at biologically relevant RNA folds.

Keywords: drug design; metal ion binding sites; molecular dynamics simulations; neomycin

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Introduction

The function of many antibiotics is based on the inhibition of a central biological process by binding of the drug to a specific RNA (Gale et al., 1981). Aminoglycoside antibiotics (Figure 1(a)) are intensively studied because they are widely used in therapy and are known to interact with a large variety of different RNA targets. The neomycins bind to functional sites in the 16 S ribosomal RNA and cause miscoding and translocation arrest of the ribosome (Moazed & Noller, 1987). Neomycin B inhibits also human immunodeficiency virus (HIV) replication in vivo by selectively blocking the binding of the Rev protein to its viral RNA target (Zapp et al., 1993). Other RNA-catalysed processes found to be inhibited by aminoglycoside antibiotics include the self-splicing of group I introns (von Ahsen et al., 1991), the cleavage reactions of the hammerhead ribozyme (Stage et al., 1995) and the hepatitis delta virus (HDV) ribozyme (Rogers et al., 1996).

Our structural understanding of specific aminoglycoside binding to RNA has recently progressed following publication of two NMR structures of aminoglycoside/RNA complexes (Jiang et al., 1996; Fourmy et al., 1996) and the results obtained by applying various techniques to study aminoglycoside/RNA interaction (Wang & Rando, 1995; Wallis et al., 1995; Werstuck et al., 1996; Famulok & Hüttenhofer, 1996; Hendrix et al., 1997; Wang & Tor, 1997a,b). Biochemical data suggest that the inhibitory effect of neomycin on the hammerhead ribozyme is based on competitive binding to the RNA between the polycationic aminoglycoside and the Mg$^{2+}$ (Clouet-d’Orval et al., 1995), which are
required for both folding and catalysis (Dahm & Uhlenbeck, 1991; Long et al., 1995). The structure of the antibiotic plays an important role in specific binding to the hammerhead RNA, since other polycations, like the polyamine spermine, do not inhibit catalysis at comparable concentrations (Dahm & Uhlenbeck, 1991).

Despite its probable biological irrelevance, we consider the inhibition of the hammerhead ribozyme a paradigm process to elucidate structural details of the interaction of a catalytical RNA with aminoglycosides for two reasons. First, because of the availability of crystal structures for this RNA (Pley et al., 1994; Scott et al., 1995, 1996). Second, considering biochemical data that suggest that, upon binding of the polycationic aminoglycosides to the hammerhead RNA, the charged ammonium groups of the antibiotic displace competitively several of the Mg\(^{2+}\) bound to the RNA (Clouet-d’Orval et al., 1995). At neutral pH five of the six amino groups in neomycin with pK\(_a\) values between 7.6 and 8.8 are protonated while the amino group at position 3 has a pK\(_a\) value of 5.7 (Botto & Coxon, 1983).

Results

Intramolecular distances between aminoglycoside ammonium groups resemble distances between Mg\(^{2+}\) binding sites in the hammerhead RNA.

We hypothesized that aminoglycoside binding to the hammerhead RNA is a structurally specific process due to a defined arrangement of charged ammonium groups in the antibiotic resembling that formed by the Mg\(^{2+}\) bound in the central pocket of the wishbone hammerhead three-dimensional structure. In order to test this hypothesis, we monitored the intramolecular distances between the amino groups in aminoglycoside antibiotics during molecular dynamics (MD) simulations and compared them with the values obtained for the interionic distances between the Mg\(^{2+}\) identified in the hammerhead crystal structure. To account for changes in the distances between amino groups due to dynamic conformational changes in the antibiotics, the accessible conformational space was explored by MD simulations on solvated amino-
glycosides at 300 K and at 600 K. For the calculations, we chose neomycin B, a 4,5-disubstituted deoxystreptamine and strong hammerhead inhibitor, and the moderate hammerhead inhibitors tobramycin and 6'-amino-6'-deoxykanamycin A, both 4,6-disubstituted deoxystreptamines (Clouetd’Orval et al., 1995; Wang & Tor, 1997a,b; Schroeder & von Ahsen, 1997; Figure 1(a)).

Similarly, we recorded the range of inter-magnesium distances observed during MD simulations of the hammerhead RNA crystal structure (Scott et al., 1996; Figure 2(a)). As described previously, the four Mg$^{2+}$ located in the cavity, formed by the facing deep grooves of stems I and II (Figure 1(b)), stayed around their binding sites in the crystal structure during MD simulations (Hermann et al., 1997). The cation at site 6 is bound to the pro-R$_p$ oxygen atom of the cleavable phosphate while the metals at the sites 1, 3 and 2 are, respectively, 7, 11 and 14 Å away from the cleavable phosphate group.

A broad range of aminoglycoside conformations was sampled during MD simulations during which inter-ammonium distances were monitored (Figure 2). The mutual orientation of rings A and B was relatively rigid in the antibiotics while, for neomycin, rings C and D displayed increased flexibility. Despite RMS deviations ranging up to 4 Å between different aminoglycoside conformers, distinct sets of constant distances between ammonium groups were systematically observed in all three drugs. An overlay plot of the recorded inter-ammonium distances on the mutual distances of Mg$^{2+}$ in the hammerhead RNA reveals a striking correspondence between pairs of cationic centers (Figure 2). Of the congruent distances, two sets are especially remarkable, namely those at 4 and 8 Å, corresponding, respectively, to the distances between Mg$_{6}^{2+}$ and Mg$_{1}^{2+}$ (∼4 Å) and between Mg$_{3}^{2+}$ and either Mg$_{6}^{2+}$ or Mg$_{1}^{2+}$ (∼8 Å). The 

Figure 2. Dynamical range of (a) interionic distances between Mg$^{2+}$ in the hammerhead RNA and ((b) to (d)) intramolecular distances between ammonium groups in (b) neomycin B, (c) tobramycin and (d) the kanamycin derivate observed during MD simulations. Along the abscissa, the inter-Mg$^{2+}$ distances from the hammerhead RNA crystal structure are marked with broken lines. (a) Distances between the Mg$^{2+}$ were recorded in simulations of the solvated hammerhead crystal structure RNA in the absence of antibiotics performed as described (Hermann et al., 1997a,b). In order to facilitate distinguishing between different Mg$^{2+}$ the bars were alternately colored. Numbering of the ions is as in Figure 1(b). Intramolecular distances between ammonium groups in the aminoglycosides (b) neomycin B, (c) tobramycin and (d) the kanamycin derivate were recorded in simulations of the solvated antibiotics. Bars indicate the dynamical range of the inter-ammonium distances (ordinate) observed at 298 K. Numbering of the amino groups is as in Figure 1(a). Ammonium distances that correspond within a narrow range to distinct inter-Mg$^{2+}$ distances are indicated in dark red. Light red bars show broad ranges of inter-ammonium distances matching several possible inter-Mg$^{2+}$ distances. Light orange bars represent inter-ammonium distances with no obvious Mg$^{2+}$ counterpart.
Mg$^{2+}$ at sites 6, 3 and 1 are the three metal ions closest to the cleavable phosphate group. In the aminoglycosides, the positively charged substituents constantly separated by around 4 Å are the two secondary amino groups 1 and 3 within ring B, positions 3 and 6$'$, and positions 3 and 2$'$ in rings A and B. Corresponding pairs of amine groups with an inter-nitrogen distance of 8 Å are found in various combinations for neomycin, between positions 1 and 2$'$, or 1 and 6$'$ for tobramycin, and between positions 1 and 6$, 1$ and 6$, or 3 and 3$'$ for kanamycin. Due to the spacer pentose inserted between the amino-substituted rings B and D in neomycin, larger distances, resembling the separation of Mg$^{2+}$ from the Mg$^{2+}$ at sites 6, 3 and 1, occur between amino groups.

Docking of solution conformations of aminoglycosides directs ammonium groups at Mg$^{2+}$ binding sites in the hammerhead RNA

To exploit the superimposition of corresponding distance pairs of ammonium groups in the aminoglycosides and Mg$^{2+}$ in the hammerhead ribozyme, we evaluated whether some of the solution conformations of aminoglycosides could be docked to the hammerhead RNA in such a way that several ammonium groups occupy simultaneously the metal binding sites of the ribozyme. We selected 16 different coordinate sets from the MD trajectory of solvated neomycin, covering the conformational space accessible to the antibiotic. For tobramycin, four conformations were picked. Each of the antibiotic solution structures was docked as a rigid molecule to the hammerhead RNA crystal structure by choosing an orientation of the aminoglycoside that resulted in an optimal fit between as many as possible ammonium groups of the drug and Mg$^{2+}$ positions in the ribozyme (Figure 3).

The resulting complexes display surprisingly good fits between the positions of the ammonium groups of the docked antibiotics and the positions of three to four Mg$^{2+}$ of the hammerhead RNA (Figure 3(b)). There was not a single docking orientation of best fit for the solution conformers of neomycin, but five different sets of ammonium/magnesium correspondences were found possible (Table 1 and Figure 3(b) and (c)). For tobramycin conformers two docking orientations were found where an optimal fit of ammonium groups to metal sites was achieved without sterical clash between the drug and the RNA. Reasons for the difference between neomycin and tobramycin are

Figure 3. (a) Representative model complex obtained by docking a solution conformation of neomycin B (blue sticks; #4 in Table 1) to the crystal structure of the hammerhead RNA. The positions of ammonium groups in the aminoglycoside that match Mg$^{2+}$ (white) are marked by red spheres. The cleavable phosphate group is indicated in yellow. (b) Several different solution conformations of aminoglycoside antibiotics resulting from MD simulations display an arrangement of ammonium groups that is complementary to the position of Mg$^{2+}$ in the hammerhead crystal structure. Overlay plots of the Mg$^{2+}$ arrangement with three different neomycin conformers and one tobramycin structure are shown. Numbers correspond to antibiotic conformers in Table 1. (c) Space-filling model of the hammerhead ribozyme with three different solution conformations of neomycin B sticks modeled by superimposing ammonium groups of the drug and Mg$^{2+}$ of the RNA crystal structure. (d) The cavity in the hammerhead RNA formed by the facing deep grooves of stems I and II, where four Mg$^{2+}$ are located, is nicely filled by aminoglycosides while the ammonium groups (red) of the drug replace the Mg$^{2+}$ (white).
the distinct substitution pattern of the deoxystreptamine moiety along with the increased flexibility of neomycin due to its pentose spacer between the amino-substituted B and D rings. Inspection of space-filling models of the complexes reveals that the different aminoglycoside conformers nicely fill the cavity formed by the facing deep grooves of stems I and II (Figure 3(c) and (d)). Surface area calculations of the hammerhead RNA alone and of the different neomycin complexes show that between 30 and 60 Å² of the van der Waals surface of the cavity formed by the facing deep grooves of the RNA complexes two classes can be distinguished. In the first class, the 2’ ammonium group of ring A occupies the Mg²⁺ binding site 3, while in the second class, this group occupies site 6 (Table 1). Two ammonium groups, namely those at position 2’ in ring A and position 1 in ring B, correspond to Mg²⁺ binding sites in all models. Interestingly, a variety of different hammerhead-inhibiting aminoglycoside antibiotics, however great their structural diversity, contain rings A and B with these two amino groups 1 and 2 (Stage et al., 1995; Wang & Tor, 1997a,b; Clouet-d’Orval et al., 1995; Schroeder & von Alshen, 1997).

Among the modeled aminoglycoside/RNA complexes two classes can be distinguished. In the first class, the 2’ ammonium group of ring A occupies the Mg²⁺ binding site 3, while in the second class, this group occupies site 6 (Table 1). Two ammonium groups, namely those at position 2’ in ring A and position 1 in ring B, correspond to Mg²⁺ binding sites in all models. Interestingly, a variety of different hammerhead-inhibiting aminoglycoside antibiotics, however great their structural diversity, contain rings A and B with these two amino groups 1 and 2 (Stage et al., 1995; Wang & Tor, 1997a,b; Clouet-d’Orval et al., 1995; Schroeder & von Alshen, 1997).

**MD simulations suggest that aminoglycoside ammonium groups mimic metal ions in their interactions with RNA**

As described above, complexes between aminoglycoside antibiotics and the hammerhead ribozyme were modeled by docking solution conformations of the drugs to the RNA in such a way that the ammonium groups overlay the Mg²⁺ binding sites. In order to study the stability of the modeled complexes and to elucidate general patterns of drug/RNA interactions, we subjected the model complexes to MD simulations. For each of the 20 different complexes a 60 ps simulation was performed in which all the non-hydrogen atoms of the RNA were fixed at their positions in the crystal structure while the docked aminoglycoside was allowed to move. By using a rigid RNA in relatively short MD trajectories, we minimized the risk of considering artefactual aminoglycoside/RNA hydrogen bonds formed only occasionally due to inaccuracies of the original docking and computational errors. However, in a longer simulation performed on one of the 20 drug/RNA complexes in which the constraints on the RNA were successively released, a constant stable binding of the aminoglycoside to the RNA was observed (data not shown).

The stability of the aminoglycoside/RNA complexes was evaluated by analysing the time-dependent hydrogen bonding patterns in the drug/RNA interaction. We differentiated between direct hydrogen bonds of aminoglycoside and RNA atoms, and hydrogen bonds mediated by a single water molecule. For neomycin, an average number of five direct and six water-mediated hydrogen bonds to the RNA were observed. Tobramycin engaged two to four direct and three to five water-mediated hydrogen bonds for RNA binding. Based on the number of interactions, two of the 16 different RNA complexes of neomycin and one of tobramycin with less than two direct hydrogen bonds between the aminoglycoside and the RNA were considered unstable.

A compilation of stable drug/RNA interactions observed during the MD simulations of the complexes is shown in Figure 4. The nucleotides of the hammerhead predominantly interacting with the drugs cluster in three regions: (i) C1’ and A1’, bordering the cleavable phosphate group; (ii) U7 and G8 in the conserved single-stranded region neighboring the U-turn; (iii) C1, C1’, U2, G2, G2, G2, and U2, consistent with earlier studies.
in the non-conserved stem I. Both the number of nucleotides involved in contacts and the character of the interactions are rather similar for the different drug/RNA complexes despite the fact that the aminoglycosides are docked to the hammerhead in several distinct orientations. The nucleotides of the above-defined single-stranded regions (i) and (ii) interact with the aminoglycosides exclusively through their backbone, predominantly through phosphate groups including the cleavable phosphate group. A single prominent exception is the N7 atom of the A1.1 base that was frequently observed forming a direct hydrogen bond to ammonium groups in the antibiotics. Of the five nucleotides within the non-conserved region (iii), U2.4 and C1.2 seem to be the most important hydrogen bonding partners for neomycin. U2.4 particularly engages its pro-RP phosphate oxygen atom while C1.2 interacts exclusively via its N4 base amino group. In some models, a direct hydrogen bridge between the O4 atom of the U2.1 base and a neomycin ammonium group was found. However, in complexes with this drug, G2.3 and G2.2 are involved in hydrogen bonding with their pro-RP phosphate oxygen atoms.

Recent NMR studies on complexes between aminoglycoside antibiotics and either an RNA aptamer and an RNA derived from the A site of 16 S rRNA, similarly reveal the importance of direct contacts between several ammonium groups of the aminoglycosides and backbone phosphate groups of the RNA (Jiang et al., 1996; Fourmy et al., 1996). Up to three ammonium groups of tobramycin were found interacting with phosphate groups of the RNA aptamer (Jiang et al., 1996). A number of similar contacts were identified in the paratomy-
in/16S rRNA complex (Fourmy et al., 1996), in line with our finding of direct contacts between hydroxyl groups of the drugs and phosphate groups of the RNA. Additional important contacts observed in our MD simulations, like the interactions between aminoglycoside ammonium groups and both purine N7 and uridine O4 atoms, were also identified in the NMR studies of drug/RNA complexes (Jiang et al., 1996; Fourmy et al., 1996).

Striking parallels were found when comparing the regions of the hammerhead RNA interacting with aminoglycoside ammonium groups in our simulations and the RNA sites responsible for Mg\(^{2+}\) binding in the hammerhead crystal structure. Of the aforementioned contacts the following RNA atoms are involved in coordination of Mg\(^{2+}\): the pro-R\(_P\) phosphate oxygen atom and N7 of A\(_{1,1}\); phosphate oxygen atoms of U\(_7\) and G\(_{8}\); O4 of the U\(_{2,1}\) base (Scott et al., 1996; Hermann et al., 1997). The similarity of aminoglycoside and Mg\(^{2+}\) binding to the hammerhead ribozyme is illustrated by a sketch of the drug/RNA interactions observed during the MD simulations of one of the modeled complexes (Figure 5). Among the most important contacts of ammonium groups and phosphate groups are those replacing the water-mediated interactions with Mg\(^{2+}\) and the cleavage-site pro-R\(_P\) phosphate oxygen atom, the direct coordination of which to Mg\(^{2+}\) was shown both by crystal structure analysis (Scott et al., 1996) and by phosphorothioate interference analysis (Knöll et al., 1997).

### Discussion

Solution conformations of the aminoglycoside antibiotics neomycin B and tobramycin could be docked to the crystal structure of a hammerhead RNA such that positively charged ammonium groups of the drugs occupy three to four Mg\(^{2+}\) binding sites of the ribozyme. MD simulations of the modeled complexes suggest that the interactions of the aminoglycosides with the RNA are almost identical to the interactions made by the hydrated Mg\(^{2+}\). Interestingly, there is not a single but several different ways to fit solution conformations of the aminoglycosides to the hammerhead metal binding sites. We propose that the structural electrostatic complementarity between the cationic groups in aminoglycosides and Mg\(^{2+}\) binding sites in folded RNAs is inherent to the arrangement of ammonium groups in aminoglycoside antibiotics. In these drugs, ammonium groups are predominantly located at distances of 4 and 8 Å due to the geometry of the linked six-membered rings. Such distances are commonly found between Mg\(^{2+}\) bound to RNA or protein enzymes involved in the formation of phosphodiester bonds, e.g., polymerases (Steitz & Steitz, 1993).

It was pointed out by Jiang and co-workers (Jiang et al., 1996) that, during their NMR study of a tobramycin/RNA aptamer complex, they were unable to identify a consistent pattern of ammonium-phosphate ionic contacts despite the fact that one to three such contacts are present in each of the refined models. This might point towards a certain diversity in the binding interaction between the aminoglycoside and the recognition pocket in the RNA. The hammerhead RNA binds aminoglycosides with lower affinity and specificity than the RNA aptamer, suggesting a less specific target for the different aminoglycoside conformers. Consequently, not a single but several different orientations of aminoglycoside conformers could be docked to the hammerhead RNA by fitting ammonium groups to metal binding sites. The diversity in the binding interaction between the drugs and the hammerhead RNA is assisted by the important number of water-mediated contacts.

The ammonium groups are singly charged and the Mg\(^{2+}\) doubly charged. However, the loss in pure electrostatic binding is compensated in the aminoglycosides by the entropic gain of providing all the charges simultaneously. Numerous additional van der Waals and hydrogen-bonding contacts can be formed by the substituents of the aminoglycosides due to the tight fitting of the drugs in the cavity between the stems I and II, as demonstrated by the burying of a substantial fraction of RNA surface upon complex formation.

The differences found experimentally (Clouet-d’Orval et al., 1995; Wang & Tor, 1997a,b) between the strong hammerhead inhibitor neomycin and the weaker inhibitor tobramycin are reflected in part by the different numbers of observed direct and water-mediated contacts between the drugs and the hammerhead RNA during the MD simulations. Clearly, we have found more stable interactions for neomycin than for tobramycin. However, care must be taken when concluding on macroscopic thermodynamic parameters from observations made on the very short time-scale of MD simulations. It has been recently shown (Wang & Tor, 1997b) that the binding strength of aminoglycosides to RNA increases with the basicity of the ammonium group, which is modulated by the adjacent hydroxyl substituents. Such subtle effects might still be beyond the limitations of the molecular mechanics approach, which uses sets of semi-empirical charges. Despite this caveat, MD simulations on solvated neomycin resulted in a greater number of conformers suitable for docking to the hammerhead RNA compared to the other two drugs. This suggests that the higher inhibiting capacity of neomycin might also be due to a greater ensemble of conformers exhibiting electrostatic complementarity to the hammerhead RNA cavity.

The principle of electrostatic complementarity presented for the hammerhead ribozyme could also give an explanation for the inhibitory effect of aminoglycosides on other catalytic RNAs, like the HDV ribozyme (Rogers et al., 1996) and group I introns (von Ahsen et al., 1991). The positions of Mg\(^{2+}\) in the three-dimensional structure of HDV ribozyme are not known and, thus, an analysis...
similar to that performed here cannot be done. However, as for the hammerhead ribozyme, the inhibition of HDV ribozyme by aminoglycosides can be competitively reversed by increasing the concentration of Mg"^{2+} (Rogers et al., 1996). Inhibition of group I intron self-splicing by aminoglycosides was detected as the first example of ribozyme inhibition by antibiotics (von Ahsen et al., 1991; Schroeder & von Ahsen, 1997). Recently, it was proposed that two Mg"^{2+} are located at a distance of 8 Å in the active site of group I introns (Streicher et al., 1996). Thus, the active site Mg"^{2+} could be replaced by cationic groups of a wide variety of aminoglycosides with their built-in inter-ammonium distances of 8 Å (I. Hoch, C. Berens, E.W., R. Schroeder, unpublished results). Interestingly, the hairpin ribozyme does not require Mg"^{2+} for catalysis (Nesbitt et al., 1997; Young et al., 1997). However, its behavior in the presence of aminoglycosides is not reported in the literature. In any case, one would not expect an effect of aminoglycosides mechanistically comparable to that seen with the hammerhead ribozyme.

Beside catalysts of the RNA world, enzymes catalyzing the polymerization of nucleic acids contain also two Mg"^{2+} at a distance of 4 to 5 Å (Steitz & Steitz, 1993; Joyce & Steitz, 1995; Sousa, 1996). This was proven by X-ray crystallographical structure determination for DNA polymerase I from Escherichia coli (Boese & Steitz, 1991) and for the DNA polymerase β from rat (Pelletier et al., 1994). Three-dimensional structures of other polymerases like the retroviral HIV-1 reverse transcriptase (Kohlstaedt et al., 1996; Jacobo-Molina et al., 1996), the bacteriophage T7 RNA polymerase (Sousa et al., 1993) and gp43, a bacteriophage DNA polymerase of the eukaryotic pol α family (Wang et al., 1997) have been analyzed without resolving the active site metals. However, the universal conservation of three aspartate residues as metal coordinating ligands in the active site of polymerases (Delarue et al., 1990) along with a structurally conserved arrangement of these aspartate residues in all polymerase structures known to date make it highly likely that the geometry of two Mg"^{2+} at a distance of approximately 4 Å occurs in all polymerases.

It was pointed out before that the fact that RNA molecules can bind aminoglycosides may indicate that these antibiotics have evolved to exploit specific recognition of nucleic acids (Davies et al., 1993; Lato et al., 1995). The occurrence of similar arrangements of Mg"^{2+} at the active sites of both ribozymes and polymerases, forming possible targets for electrostatically complementary binding of aminoglycosides, predestinates the fragments of naturally occurring aminoglycosides as versatile templates for the construction of small molecular-mass effectors. The basic building blocks found in aminoglycoside antibiotics, namely six-membered rings, carrying hydroxy and amino substituents represent tailor-made synths for the rational design of polymerase and RNA-binding inhibitors. Ammonium groups at mutual distances of around 4 Å in different structural contexts can be introduced by such single yardstick synths. The linking of different yardstick synths creates molecules carrying ammonium groups at 8 Å distances. The large variety of possible diamino-substituted six-membered rings forms an ideal basis for the application of combinatorial chemistry in the synthesis of aminoglycoside effectors (Park et al., 1996; Wang & Tor, 1997).
MD simulations of antibiotic/RNA complexes

Each aminoglycoside/RNA complex was placed in a rectangular box of SPC/E water containing about 2500 solvent molecules. Na⁺ counterions were placed according to the electrostatic potential around the complex such that no ion was closer than 4.5 Å to any solute atom. In all steps of the following calculations the non-hydrogen atoms of the RNA were fixed at their positions in the crystal structure. Initially 1000 steps of conjugate gradient minimization were performed followed by 10 ps of solvent equilibration MD at 298 K with heavy atoms of the aminoglycoside fixed. Subsequently, the constraints on the antibiotic were removed, and the system was heated from 10 K to 298 K in steps of 50 K over a period of 15 ps: 60 ps of productive MD followed. The MD trajectories were analyzed by time-averaged evaluation of hydrogen bonding between the antibiotic and the RNA.

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References


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