RNA Structures

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Binding of Aminoglycoside Antibiotics to the Duplex Form of the HIV-1 Genomic RNA Dimerization Initiation Site**

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Dimerization of the genomic RNA is a key step in retroviral replication. In HIV-1, the dimerization initiation site (DIS) is a conserved stem-loop of viral RNA containing a six-nucleotide self-complementary sequence in the loop[1] that promotes viral genome dimerization by forming a kissing-loop complex.[2] It was shown in vitro that this complex is further stabilized into an extended duplex by the viral nucleocapsid NCp7 protein[3] (Figure 1a). The stability of the dimer is strongly dependent on three purine residues flanking the self-complementary sequence.[4] Crystal structures of the DIS kissing-loop complex[5] and of the bacterial 16S ribosomal decoding site (A site) bound to aminoglycoside antibiotics[6] revealed surprising sequence and structure similarities between these two RNA structures.

As a consequence of this remarkable resemblance, 4,5-disubstituted 2-desoxystreptamine (2-DOS) aminoglycosides can also bind the DIS kissing-loop complex with a geometry similar to that observed in the A site.[7] Crystal structures of the HIV-1 subtype-F DIS kissing-loop bound to aminoglycosides were solved, thus revealing some significant differences compared to equivalent A site–aminoglycoside complexes, mainly because of the difference in topology between the two RNAs.[8,9] As a result of this, aminoglycosides exhibit a higher affinity for the DIS than for their natural target, the bacterial ribosomal A site.[10] The binding induces a strong stabilization of the kissing-loop interaction that prevents isomerization into the duplex form in vitro. Importantly, the DIS dimer remains accessible ex vivo to aminoglycoside antibiotics in HIV-1-infected lymphoid cells and in viral particles.[9]

Up to now, efforts to elucidate the potential interaction between aminoglycosides and the biologically relevant extended duplex form of the DIS RNA have failed. In the present study, we succeeded in the verification of this interaction by solving the X-ray structures of the HIV-1 subtype-F DIS duplex in the absence, as well as in the presence, of various aminoglycosides. These structures disclose the requirements for binding of aminoglycoside antibiotics to that form of the DIS, and open the way for the rationally driven design of novel potential drugs.

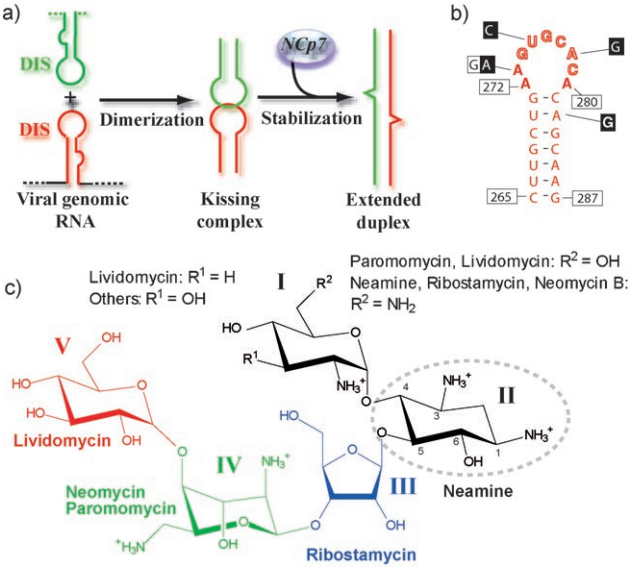

Figure 1. a) HIV-1 genomic RNA dimerization mechanism. b) RNA sequence corresponding to the HIV-1 subtype-F DIS used in this study. Sequence variations observed for HIV-1 subtypes B and A are indicated in black and white boxes, respectively. c) Chemical structures of 4,5-disubstituted 2-desoxystreptamine (2-DOS) aminoglycosides. The 2-DOS ring is circled.

We showed previously, by a comparison of X-ray structures of the HIV-1 subtype-A DIS extended duplex and kissing-loop complex, that the two DIS dimers differ not only in their topology, but also in the base-pairing pattern of the flanking purines: a G273–A280′ mismatch (the prime stands for the second strand) is observed in the extended duplex form only,[11] thus closing the "A-site motif" to drug binding. The novel structure of the unliganded subtype-F DIS duplex, obtained at 1.8 Å resolution, revealed several differences with the previously solved subtype-A DIS duplex. First, an asymmetry of bulges formed by the conserved flanking purines (adenines 272, 273, and 280; Figure 1b) is observed. In one bulge, an A272–A280′ noncanonical base pair is formed adjacent to a bulged-out A273. This differs from the G273–A280′ mismatch with bulged-out A272 observed in the HIV-1 subtype-A duplex.[11] In the second bulge, A272′ and

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A273′ are stacked in an intermediate position at the gate of the major groove, thus leaving A280 unpaired.

This conformation is clearly influenced by crystal interactions (as the bulged-out A273 of a symmetrical molecule is stacked onto A272′ and A273′) and the A272–A280′ mismatch is probably dynamic in solution. The flexibility of this region is comparable to that of equivalent adenine residues in the ribosomal A site, where A1492 (equivalent to A272 in the HIV-1 DIS) was observed either in interaction with A1408 (equivalent to A280), or flipped-out and stacked on A1493 (equivalent to A273).[12] Another difference between HIV-1 subtype-A and -F duplexes involves Mg^{2+} interaction, as only one weak binding site was observed in the present structure, whereas five strong sites were observed in the previous subtype-A structure, although crystallization conditions were identical.

The subtype-F DIS duplex was then co-crystallized with four 4,5-disubstituted 2-DOS aminoglycosides of the neamine family: ribostamycin, paromomycin, neomycin, and lividomycin (Figure 1c). These structures were solved by the multiwavelength anomalous dispersion technique using 2′-methylseleno RNA derivatives[13] at resolutions ranging from 1.5 to 2.0 Å. They reveal that, by opening the A272–A280′ base pair, the DIS duplex can also bind two aminoglycoside antibiotics per dimer, thus resulting in the A272 and A273 of both strands being clearly bulged-out.

Electron-density maps are of remarkable quality, and show in every structure two very well defined aminoglycosides (Figure 2), as well as the complete water network at the drug/RNA interface (Figure 3). At variance with the DIS kissing-loop, in which the A272 and A273 of each strand are stacked on each other,[9] the conformation of these extrahelical purine residues in the DIS duplex unexpectedly depends on the drug. In the complex with lividomycin, an A272–A273′/A273–A272′ perfect stacking is observed (Figure 2). In the presence of ribostamycin and neomycin, the stacking is restrained to A272–A273′/A272′ (with an imperfect A272–A273′ stacking) and A273 is involved in an A-minor interaction in the crystal packing. Surprisingly, in spite of these differences with the lividomycin complex, the space group and cell parameters are conserved.

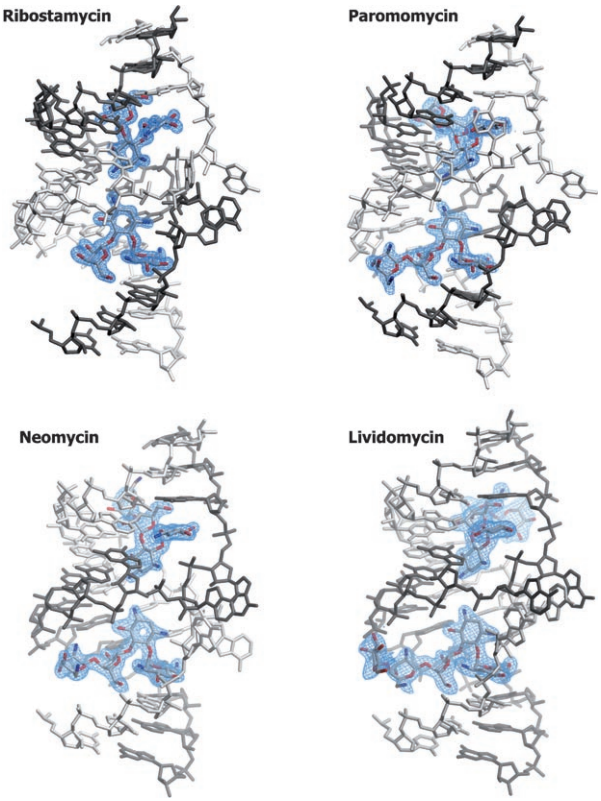

Figure 2. The four DIS duplex–aminoglycoside structures. The $2F_o-F_c$ electron-density map (F_o = observed amplitude; F_c = calculated amplitude) is shown around aminoglycosides.

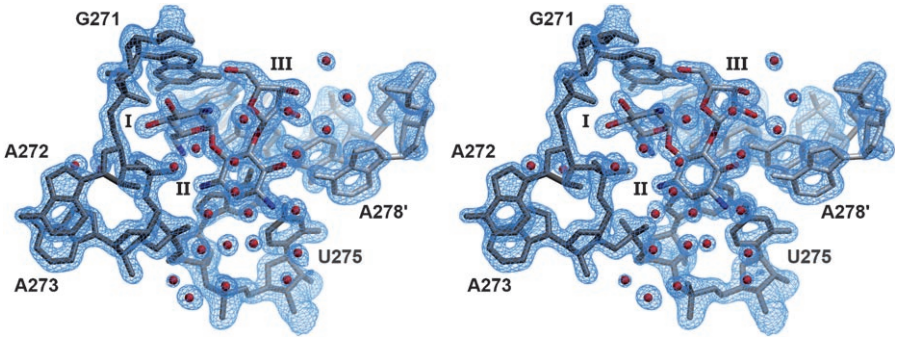

Figure 3. Stereo view of the ribostamycin–DIS duplex complex. The $2F_o-F_c$ electron-density map contoured at 1.5 σ above mean level is superimposed with the refined model. Water molecules are represented as red spheres.

By contrast, the complex with paromomycin was obtained in a different space group. In this structure, an A272–A273′/A272′ stacking is observed as well, but A273′ is bulged-out and folded back on the RNA minor groove and interacts with the G271′–C281 base pair. We cannot explain these variations of conformation observed with different aminoglycosides, as none of these drugs interacts with these positions. Apart from the high mobility of A272 and A273, the overall structures of DIS duplex–aminoglycoside complexes are very similar, especially in the A-site motif, with low root-mean-square distances (rmsd) ranging from 0.26 to 0.59 Å.

Another structural consequence of aminoglycoside binding is a distortion of the DIS duplex leading to a rmsd of 2.7–2.8 Å between bound and unbound structures. This deformation is caused by shortening and straightening of the whole helix (significantly kinked in the absence of drug) following drug binding. A similar effect was also observed, but to a lesser extent, in the DIS kissing-loop complex upon aminoglycoside binding.

Interactions between aminoglycosides and the DIS duplex are similar to contacts observed in the DIS kissing-loop complex (Figure 4). Rings I–III are responsible for the

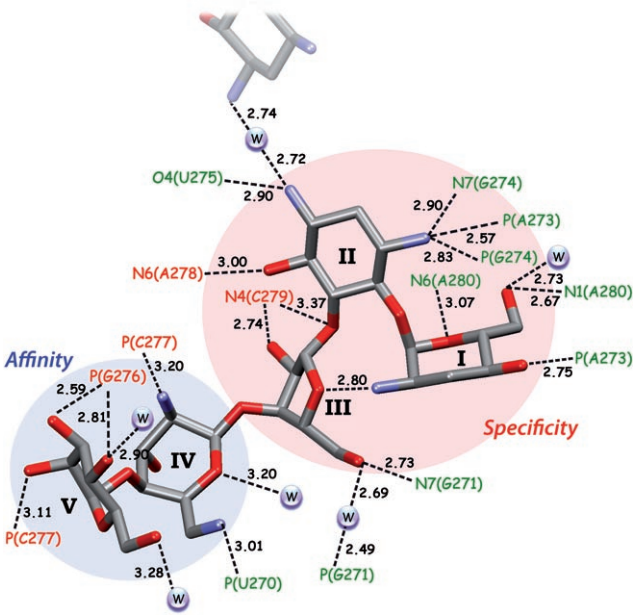

Figure 4. Lividomycin–DIS duplex contacts; "w" spheres represent water molecules. The two RNA strands are annotated in green and red. The ribostamycin core responsible for the sequence and structure specificity is circled in red, whereas rings IV and V, important for affinity in DIS–aminoglycoside complexes, are circled in blue.

structure and sequence specificity of the recognition, as direct contacts are observed with bases G271, G274, U275, and A280 in the helix major groove. Among these contacts, a pseudo-Watson–Crick base pair between A280 and ring I was observed in previous A-site and DIS kissing-loop structures in complexes with aminoglycosides. However, because of the difference in RNA topology, the total number of direct contacts in DIS duplex–aminoglycoside complexes is reduced compared to their DIS kissing-loop–aminoglycoside counterparts (that is, ten contacts instead of 13 for each DIS–lividomycin complex). The missing contacts involve A272 and A273 phosphates in the duplex topology. Likewise, a potassium cation specific to aminoglycoside–DIS kissing-loop complex interaction[8,9] is not observed in the present structures. As a consequence, the surface area buried at the drug/RNA interface for the duplex form is reduced on average by 135 Å^2 compared with the kissing-loop form.[8] For example, this surface area is 1044 Å^2 for the complex with lividomycin in the duplex form and 1209 Å^2 in the loop–loop complex. From this finding, it may be presumed that the affinity of aminoglycoside for the DIS duplex is lower than that for the kissing-loop complex.

Notwithstanding these differences, a comparison with previous DIS kissing-loop and ribosomal A-site structures, as well as among DIS duplex structures, showed that the "ribostamycin core" formed by rings I–III is remarkably rigid. As a consequence, the ligand is responsible for an induced fit of the RNA A-site motif, which leads to similar structures despite the difference in RNA topology. Indeed, we observed a drop of the rmsd between A-site motifs in both topologies from 1.9 Å with unbound structures to roughly 0.8 Å with aminoglycoside complexes. The same is true for the rmsd of the overall structures, with a drop from 2.5 to 1.3 Å.

At variance with the rigid ribostamycin core, rings IV and V, which were shown to be essential for affinity to the DIS kissing-loop form,[10] exclusively interact with the RNA backbone through unspecific contacts (Figure 4). These two rings are in a different orientation compared to their counterparts in DIS kissing-loop complexes, but are perfectly ordered in electron-density maps of all DIS duplex structures (Figure 2). This contrasts with kissing-loop and A-site structures,[6,14] where rings IV and V were characterized by a significant mobility (high *B* factors as a result of alternating positions or disorder).

We have previously determined the thermodynamic parameters of aminoglycoside–DIS kissing-loop interactions, and showed that these drugs exhibit a high affinity for the kissing-loop complex (down to 32 nM) under our experimental conditions.[10] The present structures provide evidence that aminoglycoside antibiotics can also bind the DIS extended duplex form, in addition to the initial DIS kissing-loop complex form. Importantly, this finding shows that it is possible to target the HIV-1 genomic RNA before and after its maturation by the NCp7 nucleocapsid protein with small molecules, such as aminoglycoside antibiotics, and even with the same drug. Alternatively, specific molecules might be designed to distinctively bind both RNA topologies by using, for instance, the different contacts specific for the RNA topology involving ring I, or the variation in the orientation of rings IV and V. These differences might also be taken into account in the frame of the development of aminoglycoside dimers that could specifically recognize the DIS kissing-loop or extended duplex forms.[15]

Previous studies reported that aminoglycosides could inhibit HIV-1 production (up to 85%) in a dose-dependent manner in infected U1 cells,[16] but not on CEMSS or MT4 cell lines.[9] To clarify these conflicting results, the impact of aminoglycoside binding on viral replication will now be further investigated in vitro. As the viral reverse transcriptase (RT) enzyme has to dissociate RNA secondary and tertiary structures to achieve synthesis of the proviral DNA, our efforts will be particularly focused on the drug-induced stabilization of the viral RNA dimer that might induce pauses of the RT,[10] and thus interfere with virus production.

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