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Crystal structures of Homo sapiens cytoplasmic A site with and without the antibiotic apramycin

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All aminoglycoside antibiotics are toxic to human and some of these effects have been attributed to their binding to the *Homo sapiens* cytoplasmic or mitochondrial A site due to their sequence similarities with the bacterial A site. To understand the mechanisms of aminoglycoside toxicity at the atomic level, we have solved the crystal structures of RNA fragment containing two *H. sapiens* cytoplasmic A sites with and without the aminoglycoside apramycin at 2.8 Å and 2.3 Å resolutions, respectively [1, 2]. In the RNA crystal without apramycin, the two A sites are in different conformational states, the ''on'' and ''off'' states. In the ''on'' state, two adenine residues A1492 and A1493 are fully bulged out and A1491 forms a wobble-like pair with C1409. Therefore, the conformation of the ''on'' state is basically the same as in the bacterial A site with bulging A1492 and A1493. On the other hand, the ''off'' state with A1492oC1409 and A1493oG1408 base pairs and bulged-out A1491 is drastically different from any of those observed for the bacterial A site without bulged-out A1492 and A1493. In the RNA/apramycin complex crystal, apramycin specifically binds to the deep/major groove of the cytoplasmic A site. The binding mode of apramycin to the cytoplasmic A site is surprisingly different from that observed in the bacterial A site complex [3]. Although apramycin binds to the ''on'' state of the bacterial A site, it binds to the ''off'' state of the *H. sapiens* cytoplasmic A site. Comparative structural analysis shows that apramycin uses different interaction modes, (i) the A1408 (bacterial) and G1408 (cytoplasmic) residues for the interactions between ring I and residue 1408; (ii) the C1409=G1491 (bacterial) and C1409oA1492 (cytoplasmic) pairs for the triple interactions with ring III. Superimposition between the *H. sapiens* cytoplasmic A site in complex with apramycin and crystal structure of the 30S ribosome in complex with mRNA, tRNA and paromomycin [4] yields some insight into the molecular mechanisms of apramycin toxicity to human. The bulged-out A1493 residue, which monitors the first Watson-Crick base pair of the codon-anticodon helix, protrudes toward the mRNA-tRNA complex. However, it is too far from the codon-anticodon base pair to form an A-minor motif. Since A1492 is inside the A site helix, it cannot recognize the second Watson-Crick base pair of the codon-anticodon helix. In addition, the bulged-out A1491 residue is in close contact with ribosomal protein S12, which is known as a control element for translocation of the mRNA-tRNA complex. Therefore, in the *H. sapiens* 40S cytoplasmic A site, the A1491 residue might disturb the local conformation of a cytoplasmic ribosomal protein thereby inhibiting translocation of the *H. sapiens* cytoplasmic ribosome.

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Conformational variability of DNA and comparison to RNA

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The number of nucleic acid structures deposited in the NDB [1] allows their detailed conformational analysis. We investigated conformations of more than eight thousand nucleotides from over four hundred well resolved DNA x-ray structures and analyzed their conformations by a combination of the Fourier averaging method and clustering techniques developed previously [2]. The known double helical conformations, A-DNA, BI- and BII-DNA, as well as the Z forms, have many well defined conformational substates that are localizable in x-ray structures. Many substates exist also for conformations that represent A-to-BI as well as for BI-to-BII transitions. Some less common but distinct conformations were observed only in protein/DNA complexes, others in drug/DNA complexes, some are characteristic by specific combinations of torsion angles, as parallel tetraplex by both α and γ in the gauche+ region. Perhaps surprisingly, 1D and 2D distributions of torsion angles of both forms of nucleic acids look similar and the double helical A and B conformations represent majority of their populations. The main structural difference between DNA and RNA seems to be in the way how individual conformations relate to each other. Substates in the DNA conformational space are close to each other, transitions between them are almost continuous, form paths, and deformations of DNA molecules can therefore be realized in small steps. In contrast, widely diverse RNA conformations seem to form isolate islands in the conformational space and transformation from one state to another has to cross larger unpopulated areas.

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