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Aminoglycoside Recognition in the Apramycin Complex of Eukaryotic Ribosomal Decoding Site RNA

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Apramycin stands out for its unique bicyclic core structure among aminoglycoside antibiotics that cause misreading of the genetic code during translation by binding to the ribosomal decoding site RNA. We have determined the crystal structure of a complex between an oligoribonucleotide containing the eukaryotic decoding site and apramycin at 1.5 Å resolution. Crystals of the RNA-apramycin complex grew at 20°C over 3-4 days by vapor diffusion in hanging drops mixed from 2 micro l 0.32 mM complex and 2 micro l of precipitant buffer containing 50 mM MES (pH 5.6), 18-19% MPD, and 20-40 mM Mg acetate. Prior to freezing for X-ray analysis, crystals were briefly soaked in precipitant buffer containing 30% MPD. The crystals belong to the P212121 space group with unit cell dimensions $a = 28.31 \text{ \AA}$, $b = 36.69 \text{ \AA}$, $c = 86.68 \text{ \AA}$. The structure was solved by molecular replacement with use of AmoRe program and refined with Refmac program. There are one complex, two Mg ions, one sulfate ion and 172 water molecules in asymmetric unit. R-factor is 19.9% (R-free=22.7%) using all data with $F > 0$.

The drug binds in the deep groove of the decoding site RNA which forms a continuously stacked helix comprising novel non-canonical C•A and G•A base pairs and a bulged adenine. Apramycin recognizes the RNA target by specific direct contacts and interactions mediated by a Mg^{2+} ion and water molecules. The complex structure reveals for apramycin a mode of binding to the eukaryotic decoding site that is distinct from aminoglycoside recognition at the bacterial ribosome, suggesting a molecular basis for the distinct actions of apramycin in eukaryotes and bacteria.

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