Mapping the interactions of selected antibiotics and their Cu^{2+} complexes with the antigenomic delta ribozyme.

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Streszczenie

The interactions of selected antibiotics with the *trans*-acting antigenomic delta ribozyme were mapped. Ribozyme with two oligonucleotide substrates was used, one uncleavable with deoxycytidine at the cleavage site, mimicking the initial state of ribozyme, and the other with an all-RNA substrate mimicking, after cleavage, the product state. Mapping was performed with a set of RNA structural probing methods: P b^{2+} -induced cleavage, nuclease digestion, and the selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) approach. The experimental results combined with molecular modeling revealed different binding sites for neomycin B , amikacin and actinomycin D inside the ribozyme structure. Neomycin B , an aminoglycoside antibiotic, which strongly inhibited the catalytic properties of delta ribozyme, was bound to the pocket formed by the P 1 stem, the P 1.1 pseudoknot, and the J 4/2 junction. Amikacin showed less effective binding to the ribozyme catalytic core, resulting in weak inhibition. Complexes of these aminoglycosides with C u^{2+} ions were bound to the same ribozyme regions, but more effectively, showing lower K_d values. On the other hand, the C u^{2+} complex of the cyclopeptide antibiotic actinonomycin D was preferentially intercalated into the P 2 and the P 4 double-stranded region, and was three times more potent in ribozyme inhibition than the free antibiotic. In addition, some differences in SHAPE reactivities between the ribozyme forms containing all-RNA and deoxycytidine-modified substrates in the J 4/2 region were detected, pointing to different ribozyme conformations before and after the cleavage event.

Słowa kluczowe

antibiotics, delta ribozyme, modeling ofantibiotic–ribozyme interaction, RNAprobing, RNA structure