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The Structures of four Macrolide Antibiotics Bound to the Large Ribosomal Subunit

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Introduction: Since the discovery of streptomycin in the 1940s, thousands of low molecular weight compounds have been found that inhibit protein synthesis (Vazquez, 1979). A number of them are clinically useful because, like streptomycin, they inhibit protein synthesis in bacteria far more effectively than in mammals. Prominent among this subset of protein synthesis inhibitors are the macrolides, a large family of both natural and semisynthetic antibiotics, all of which consist of 14- to 16-membered lactone rings to which sugar substituents are attached. Macrolides bind to the large ribosomal subunit and inhibit protein synthesis (Gale et al.).

Methods and Materials: Ribosomes were purified and crystallized as described previously (Ban et al., 2000). Antibiotics were selected for testing based on their known activity against *Haloarcula marismortui* (Sanz et al., 1993) and based on availability. Antibiotics were solubilized in dimethylsulfoxide (DMSO), then added to the standard stabilization buffer (Ban et al., 2000 \star) to a final concentration of 1.0 to 10.0 mM (and a final DMSO concentration of 1 to 4%), and incubated at 4°C for 24 hr prior to cryo-vitrification of crystals in liquid propane. Initial X-ray diffraction data were collected at beamlines X25, X12b, or X12c at Brookhaven. Electron density corresponding to these macrolides was first seen in $F_o(\text{antibiotic})-F_o(\text{native})$ difference Fourier maps at 4.0 Å resolution. Higher resolution data were collection at beamline ID19 at the Advanced Photon Source, Argonne National Laboratory. The antibiotic models were initially fit into F_o-F_o difference electron density maps. Coordinates are available at the Protein Data Bank with accession numbers 1K8A, 1K9M, 1M1K, and 1KD1.

Results: Crystal structures of the *Haloarcula marismortui* large ribosomal subunit complexed with the 16-membered macrolide antibiotics carbomycin A, spiramycin, and tylosin and a 15-membered macrolide, azithromycin, show that they bind in the polypeptide exit tunnel adjacent to the peptidyl transferase center. Their location suggests that they inhibit protein synthesis by blocking the egress of nascent polypeptides. The saccharide branch attached to C5 of the lactone rings extends toward the peptidyl transferase center, and the isobutyrate extension of the carbomycin A disaccharide overlaps the A-site. Unexpectedly, a reversible covalent bond forms between the ethylaldehyde substituent at the C6 position of the 16-membered macrolides and the N6 of A2103 (A2062, *E. coli*). Mutations in 23S rRNA that result in clinical resistance render the binding site less complementary to macrolides.

Conclusions: Macrolides bind at the narrow opening to the peptide exit tunnel where, as expected, they would block an elongating peptide. A covalent bond links A2103 of ribosomal RNA to those macrolides that have an aldehyde group at C6. Hydrophobic interactions dominate the binding of one face of the 15- and 16-membered lactone rings to the ribosome. One common hydrogen bond connecting O2A of mycaminose to N1 of G2099 (A2058) is observed in all structures of macrolide-ribosome complexes. The disaccharide branch attached to the C5 of the lactone ring extends toward the peptidyl transferase center, so that macrolides with long branches from C5 more directly inhibit the peptidyl transferase reaction than those with shorter branches. These structures may assist in the design of new antibiotics that will work against the presently known resistance mutations.

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