Crystal Structure of a Bioactive Pactamycin Analog Bound to the 30S Ribosomal Subunit

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Abstract

Biosynthetically and chemically derived analogs of the antibiotic pactamycin and de-6-methylsalicylicyl (MSA)-pactamycin have attracted recent interest as potential antiprotozoal and antitumor drugs. Here, we report a 3.1-Å crystal structure of de-6-MSA-pactamycin bound to its target site on the Thermus thermophilus 30S ribosomal subunit. Although de-6-MSA-pactamycin lacks the MSA moiety, it shares the same binding site as pactamycin and induces a displacement of nucleic acid template bound at the E-site of the 30S. The structure highlights unique interactions between this pactamycin analog and the ribosome, which paves the way for therapeutic development of related compounds.

Antibiotics that bind selectively to bacterial or protozoal ribosomes are of great clinical significance due to their ability to treat infectious diseases without compromising the host.1,2 The most effective antibiotics used in clinical treatment exploit subtle differences between distinct locations within the functional sites of prokaryotic and eukaryotic ribosomes. On the other hand, compounds such as sparsomycin3 and pactamycin,4 which are known to interact with the ribosome with universal specificity, have been reported as potential antitumor drugs.

The aminocyclopentitol pactamycin (Fig. 1a) was first isolated from Streptomyces pactum as a potential antitumor drug and later found to exhibit potent activity against many bacteria, archaea, and eukaryotes.4,5 In accordance with biochemical data,6,7 the crystal structure of pactamycin bound to the 30S ribosomal subunit revealed that this antibiotic binds near a highly conserved region of 16S RNA at what is now known to be ribosomal E-site.8 It was therefore proposed that pactamycin prevents a codon–anticodon interaction forming at this location and blocks the translocation of P-site tRNA into the E-site of the 30S.9

The biosynthetic pathway of pactamycin has been elucidated10 and shown to proceed via an intermediate compound, de-6-methylsalicylicyl (MSA)-pactamycin (Fig. 1a). This compound lacks the 6-methylsalicylic acid ring of the parent molecule and yet displays equivalent antibacterial and antitumor activity to pactamycin, suggesting that the 6-methylsalicylic acid moiety is not required for cell toxicity.10 Biosynthetic products related to de-6-MSA-pactamycin also inhibit growth of malarial parasites, but with a significant reduction in toxicity to mammalian cells.11,12 Likewise, semisynthetic analogs of de-6-MSA-pactamycin, prepared following the first total synthesis of pactamycin13 and varying in the nature of the urea or the aniline moieties, exhibit potent in vitro antiparasitic and antitumor activity.14 A recent enantioselective synthesis of pactamycin totaling only 15 steps will augur well for newer analogs.15,16

Knowing that de-6-MSA-pactamycin maintained its in vitro antibacterial, antitumor, and antiparasitic activities, we were particularly interested to see how the absence of the 6-methylsalicylic acid moiety would affect its binding to the ribosome. Consequently, we determined the crystal structure of the Thermus thermophilus 30S ribosomal subunit bound to de-6-MSA-pactamycin in the presence of paromomycin, which enables a detailed description of interactions between pactamycin analogs and the ribosome. Following refinement of the initial atomic model, we
unambiguously placed de-6-MSA-pactamycin into electron density identified at the tip of helix 23b (Fig. 1b). This location has previously been described as the binding site of pactamycin.8 The two distal aromatic rings of pactamycin are known to stack against each other and G693 of helix 23b due to the antibiotic adopting a folded structure mimicking an RNA dinucleotide. This was suggested to result in a displacement of the E-site mRNA. Similarly, the remaining aminoacetophenone moiety of de-6-MSA-pactamycin stacks against the base of G693, where it is stabilized by O6 and N7 forming hydrogen bonds with an amine and ketone on the neighboring cyclopentitol.

A superposition of our structure with the empty 30S subunit reveals that, like pactamycin,8 de-6-MSA-pactamycin prevents the 3′ end of 16S RNA from folding back on itself to mimic an E-site codon. However, the absence of a 6-methylsalicylic acid moiety on de-6-MSA-pactamycin means that the 3′ end of the 16S, and presumably the path of mRNA, is displaced to a lesser extent than it would be in the presence of pactamycin (~8.0 Å compared to ~12.5 Å; Fig. 1b). This allows base U1540 of the 16S to form a novel hydrogen bond via its O2 and the carbonyl group of the aminoacetophenone ring (interaction 1; Fig. 1c). Interestingly, replacement of the acetyl group in the aniline moiety of de-6-MSA-

![Diagram](image-url)
pactamycin by fluorine or trifluoromethyl results in potent in vitro antimalarial activity. It is likely that a hydrogen bond is shared between fluorine and U1540 when such compounds form a complex with the ribosome.

Further hydrogen bond interactions were identified between bases G693 and C796 and functional groups on the extensions of the central ring (Fig. 1c, interactions 2–6). The N4 of base C795 forms a hydrogen bond with the hydroxyl group on the C7 cyclopentitol atom (interaction 6; Fig. 1c), which is absent in the antimalarial analog de-6-MSA-7-deoxypactamycin. It would therefore appear that a loss of this hydrogen bond is sufficient to reduce binding of de-6-MSA-7-deoxypactamycin to the mammalian ribosome, enough to lower cell toxicity 10- to 30-fold. Together, these interactions mean that de-6-MSA-pactamycin forms a tightly bound complex with the ribosome that disrupts base pairing at the E-site of the 30S subunit.

Although de-6-MSA-pactamycin shares the same binding site as pactamycin, a new collection of antibiotic–ribosome contacts distinguishes this derivative from its parent molecule. A complete understanding of such interactions will aid in the design of new and improved analogs toward the development of effective antiprotozoal and antitumor drugs.

Acknowledgements

We would like to thank the beamline staff on IO4 at Diamond Light Source for help and advice with data collection. This work was supported by grants to V.R. from the UK Medical Research Council (U105184332), the Wellcome Trust, the Agouron Institute, and the Louis-Jeantet Foundation. Financial assistance from Natural Sciences and Engineering Research Council of Canada and Fonds de Recherche du Québec Nature et Technologies is acknowledged (S.H.).

Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jmb.2013.05.004

Keywords: translation; antibiotic; E-site; mRNA

†D.S.T. and I.S.F. contributed equally to this work.

Abbreviation used:
MSA, methylsalicylal.

References


Supporting Information

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Materials and Methods

The 30S ribosomal subunit from Thermus thermophilus was purified and crystallized using the method described previously. Crystals of diffraction quality were transferred to a cryo-protectant [100 mM Mes-KOH (pH 6.5), 200 mM KCl, 75 mM NH4Cl, 15 mM MgCl2, and 26% (vol/vol) 2-Methyl-2,4-pentanediol (MPD)] containing a mixture of 1 mM de-6-MSA-pactamycin and 100 µM paromomycin to improve resolution. Diffraction data were collected from a single crystal that diffracted beyond 3.1 Å on the IO4 beamline at the Diamond Light Source, Harwell, England.

Diffraction images were integrated and scaled using the XDS package prior to a round of restrained refinement in REFMAC5 with the empty 30S structure as a starting model. Each initial refinement was followed by alternating cycles of model building in COOT and automated refinements using jelly-body restraints in REFMAC5. At each stage of refinement...
electron density for ligands could be clearly identified in the unbiased difference maps, but ligand atomic coordinates were not included until the final round of refinement where a de-6-MSA-pactamycin molecule and seven paramomycin molecules were placed with confidence into the electron density map. The final model had an $R_{\text{work}}/R_{\text{free}}$ ratio of 18.4/22.7.

ACCESSION NUMBERS: Coordinates and structure factors have been deposited in the Protein Data Bank with accession code 4KHP.

**Table 1.** Summary of crystallographic data and refinement.

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<thead>
<tr>
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<th>30S + de-6-MSA-pactamycin + paromomycin</th>
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<td>Beamline</td>
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<td><strong>Cell dimensions</strong></td>
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<tr>
<td>$\alpha, \beta, \gamma$ (°)</td>
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<td>Bond angles r.m.s.d. (°)</td>
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</table>

* $I/\sigma(I) = 2.05$ at 3.2 Å (using a bin from 3.3 – 3.2 Å resolution).

**References**

