

Bactobolin A Binds to a Site on the 70S Ribosome Distinct from Previously Seen Antibiotics

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Abstract

The ribosome is the target of a large number of antibiotics. Here, we report a 3.4-Å-resolution crystal structure of bactobolin A bound to 70S ribosome–tRNA complex. The antibiotic binds at a previously unseen site in the 50S subunit and displaces tRNA bound at the P-site. It thus likely has a similar mechanism of action as blasticidin S despite binding to a different site. The structure also rationalizes previously identified resistance mutations.

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Structural studies have been instrumental in revealing the molecular basis of the action of antibiotics [1]. Those studies showed that many antibiotics inhibit various steps of the elongation cycle of translation [2], whereas relatively few clinically useful compounds target initiation [3]. Recently, it was suggested that blasticidin S (BlaS) acts by inhibiting termination [4].

Bactobolin is a member of the polyketide-peptide family of molecules produced by *Burkholderia thailandensis* [5]. These water-soluble compounds consist of a C₆-polyketide fused to a chlorinated hydroxy-valine residue (Fig. 1a). Recently, it was shown to inhibit protein synthesis, and resistance to bactobolin is acquired by mutations in the 50S ribosomal protein uL2 [6]. Bactobolin-resistant mutants remain susceptible to other known ribosome inhibitors, suggesting that it binds to a novel target site in the ribosome.

To help understand the molecular basis of its action, we determined the crystal structure of bactobolin A bound to the *Thermus thermophilus* 70S ribosome in the presence of mRNA and tRNA. The resolution of 3.4 Å allowed us to visualize the antibiotic and deduce its interactions with the ribosome and tRNA. Following refinement of the initial model, we placed bactobolin A in the electron density (Fig. 1b and c). The antibiotic is coordinated primarily by helix 73, and its interactions with the ribosome involve a Mg²⁺ ion that allows it to interact with A2613 through both of its rings via the

carbonyl oxygen of the lactone and the hydroxyl of the enol (Fig. 1c). The additional enol hydroxyl is bound by C2085, whereas the chlorides of the lactone ring are coordinated by U2449, A2611 and C2612. The binding of bactobolin is further stabilized by the amine termini of hydroxy-valine interacting with A2450. This holds bactobolin in the orientation that results in direct contacts with the CCA end of P-site tRNA (Fig. 1c and d).

A superposition of our structure with a 70S–tRNA complex without the antibiotic shows that bactobolin would clash sterically with canonical P-site tRNA (Fig. 1d). Thus, in order to accommodate both tRNA and the antibiotic, the CCA backbone of P-site tRNA is displaced in our structure (Fig. 1d). Consequently, A76 of P-tRNA no longer contacts C2063 and is shifted 3.6 Å toward the A-site. Such a conformational change of the P-site tRNA caused by bactobolin A suggests that it would inhibit peptidyl transfer. However, the structural effect of bactobolin A binding is similar to the one recently reported for BlaS [4]. It has been further shown that BlaS-stimulated distortion of CCA P-tRNA would occlude the access of a release factor to the A-site and thus inhibit translation termination, which is also supported by biochemical data [4]. The fact that BlaS and bactobolin A have overlapping binding sites (Fig. 1b) and cause a similar conformational rearrangement of P-site tRNA suggests that both act as translation termination inhibitors.

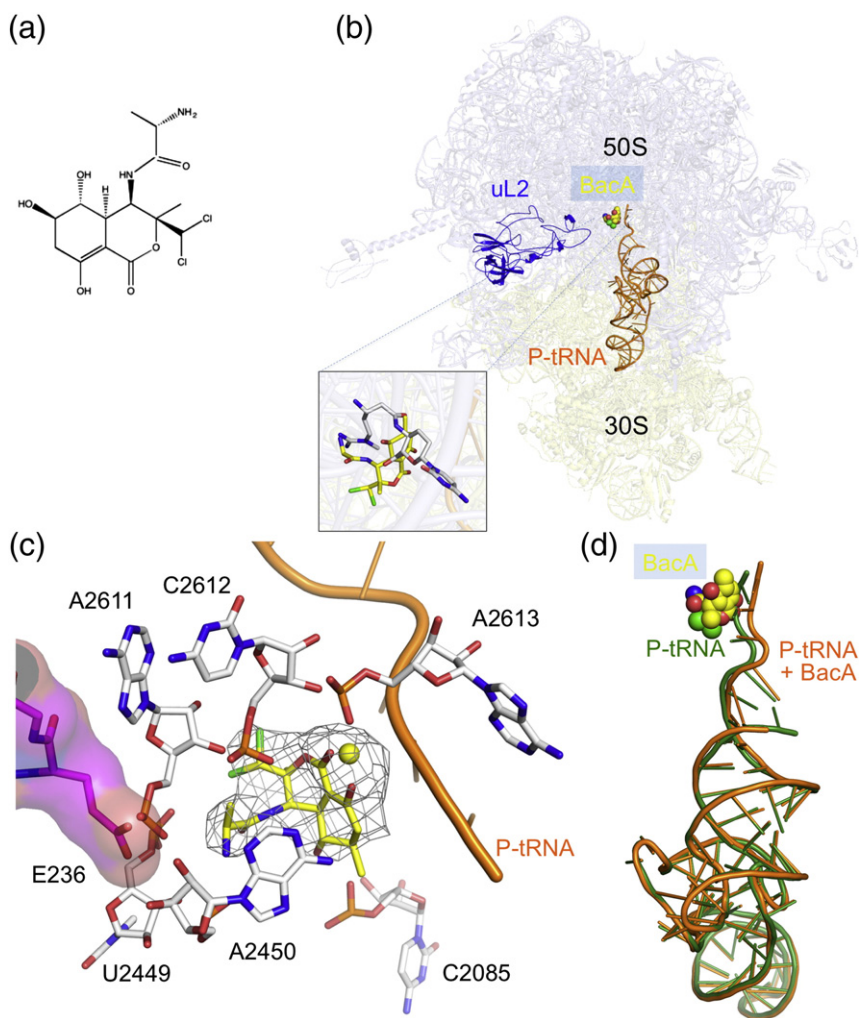


Fig. 1. Binding of bactobolin A to the ribosome in the presence of P-tRNA. (a) Chemical structure of bactobolin. (b) Relative positions of bactobolin, tRNA and uL2; overlapping of bactobolin and BlaS binding sites. (c) Unbiased $F_o - F_c$ difference map corresponding to bactobolin A and adjacent Mg^{2+} ion (yellow sphere) is contoured at 3σ . rRNA forming contacts with bactobolin is shown. E236 of uL2 stabilizes A2450. (d) Superposition of tRNA in the 70S-tRNA-bactobolin complex with that from antibiotic-free 70S-tRNA, demonstrating the shift of the CCA end toward the A-site.

The crystal structure also rationalizes biochemical data that identified a mutation in E236 (*Bacillus subtilis*) of uL2 protein as a cause of resistance to bactobolin [6]. The resistance can be explained by disruption of E236:A2450 contacts (Fig. 1c). Since A2450 is directly involved in coordinating bactobolin A, the mutation is likely to disorder the binding site. We note that the previously described bactobolin-resistant mutants remain susceptible to BlaS.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jmb.2014.12.018>.

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BlaS, blasticidin S.

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