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Research paper

Interactions of amikacin with the RNA model of the ribosomal A-site: Computational, spectroscopic and calorimetric studies

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ABSTRACT

Amikacin is a 2-deoxystreptamine aminoglycoside antibiotic possessing a unique L-HABA (L-(-)-γ-amino-α-hydroxybutyric acid) group and applied in the treatment of hospital-acquired infections. Amikacin influences bacterial translation by binding to the decoding region of the small ribosomal subunit that overlaps with the binding site of aminoacylated-tRNA (A-site). Here, we have characterized thermodynamics of interactions of amikacin with a 27-mer RNA oligonucleotide mimicking the aminoglycoside binding site in the bacterial ribosome. We applied isothermal titration and differential scanning calorimetries, circular dichroism and thermal denaturation experiments, as well as computer simulations.

Thermal denaturation studies have shown that amikacin affects only slightly the melting temperatures of the A-site mimicking RNA model suggesting a moderate stabilization of RNA by amikacin. Isothermal titration calorimetry gives the equilibrium dissociation constants for the binding reaction between amikacin and the A-site oligonucleotide in the micromolar range with a favorable enthalpic contribution. However, for amikacin we observe a positive entropic contribution to binding, contrary to other aminoglycosides, paromomycin and ribostamycin. Circular dichroism spectra suggest that the observed increase in entropy is not caused by structural changes of RNA because amikacin binding does not destabilize the helicity of the RNA model. To investigate the origins of this positive entropy change we performed all-atom molecular dynamics simulations in explicit solvent for the 27-mer RNA oligonucleotide mimicking one A-site and the crystal structure of an RNA duplex containing two A-sites. We observed that the diversity of the conformational states of the L-HABA group sampled in the simulations of the complex was larger than for the free amikacin in explicit water. Therefore, the larger flexibility of the L-HABA group in the bound form may contribute to an increase of entropy upon binding.

1. Introduction

The most known role of RNA is to provide intermediates for translation in the form of messenger RNA. However, a plethora of noncoding, but nevertheless functional, RNAs has been described over the recent years [1]. Understanding their dynamics at the atomistic level is a challenge that can be faced only by combining experimental and computational studies [2]. Due to diverse roles, RNA is a promising target for inhibitors that control RNA expression or modulate its function. In particular, many antibiotics target ribosomal RNA (rRNA) and inhibit protein synthesis in bacteria [3]. One class of such antibiotics are aminoglycosides, which have been administered clinically since 1943, and despite adverse effects are still the antibiotics of choice used in hospitals [4]. The examples of diseases treatable with aminoglycosides include tuberculosis, conjunctivitis and systemic infections [5].

Many aminoglycosides, which include the 2-deoxystreptamine (2-DOS) core, bind to the rRNA of the small subunit of the bacterial ribosome (namely 16S rRNA). Their binding site overlaps with

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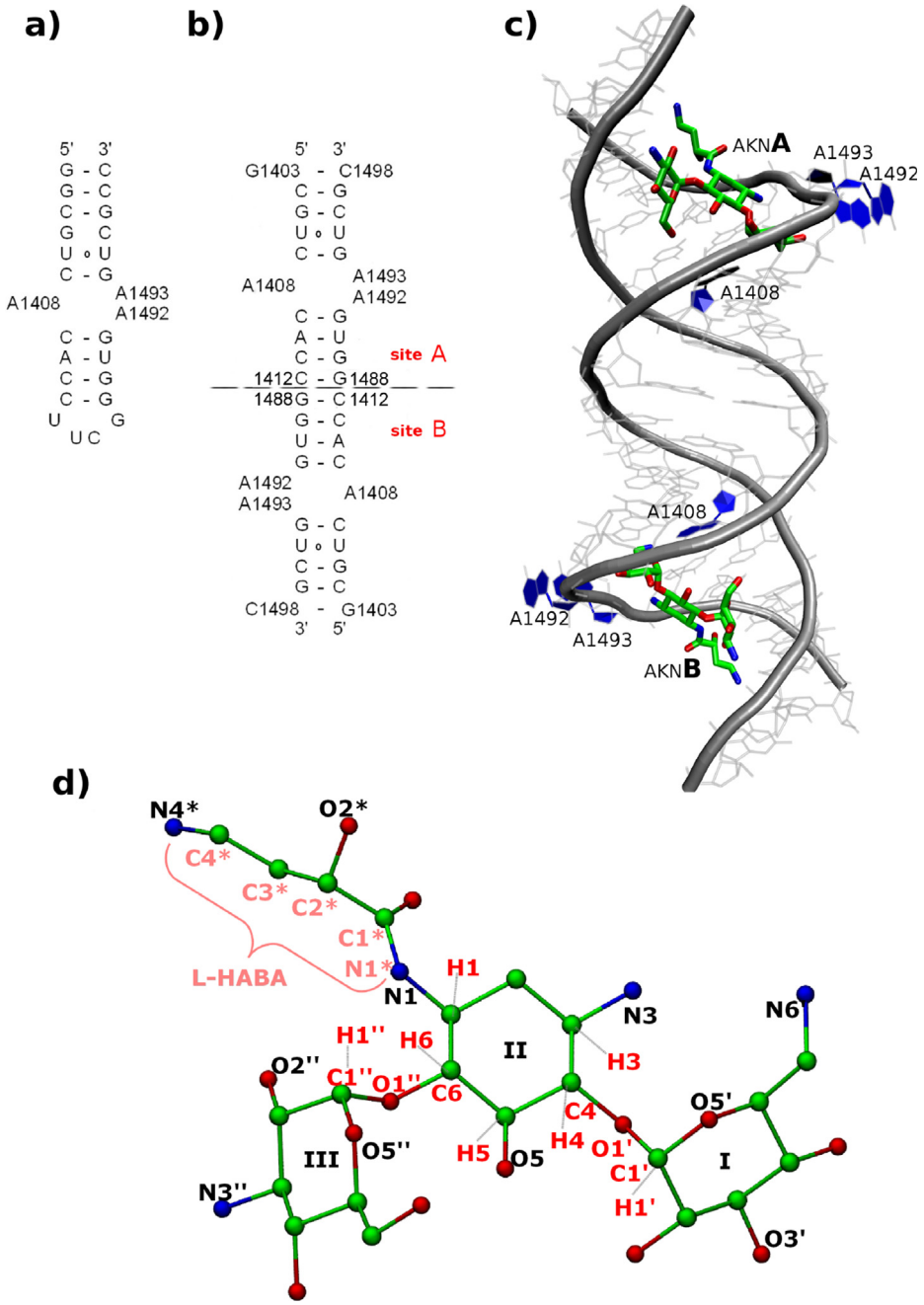

Fig. 1. a) The RNA oligonucleotide containing one A-site used in the experimental measurements and MD simulations, labeled RNA-loop and RNA-loop–AKN; b) the sequence of the MD simulated crystal structure containing two A-sites (labeled RNA and RNA–AKN), the boxes denote two mirrored amikacin binding sites, referred to as sites A and B; c) visualization of the crystal structure of the model from panel b), with two bound amikacins AKNA and AKNB (PDB ID: 2G5Q) [13]; d) heavy atom model of the chemical structure of amikacin with the atoms discussed in the text labeled.

the site that binds aminoacylated-tRNA, called the A-site [6]. It forms a bulge in the RNA helical structure, with two nucleotides, A1492 and A1493, on one strand and A1408 on the opposite strand (Fig. 1a–c). The binding of 2-DOS aminoglycosides to 16S rRNA is intrinsically connected with flipping of A1492 and A1493 outside the A-site rRNA helical bulge and acquiring extra-helical states [7,8]. This flipping event has been previously studied and provided information on the binding site geometries [9,10], the indirect (water-mediated) [11,12] and direct hydrogen bonds between rRNA and aminoglycosides [13]. Moreover, certain nucleotides in the A-site sequence (e.g., A1408) are critical for aminoglycoside binding because they interact with particular rings of the drug [14–18]. It was shown that mutations in this rRNA region can lead to resistance against aminoglycosides [19–22].

The thermodynamic binding data for a variety of 2-DOS aminoglycosides associating with a model of the 16S rRNA A-site and its variants have been determined using fluorescence spectroscopy, surface plasmon resonance, isothermal titration calorimetry, and thermal denaturation studies [23–31]. Overall, the equilibrium dissociation constants are in the micromolar range. The binding affinities correlate with the net charge of aminoglycoside and the binding above pH 5.5 is linked to the uptake of protons by the drug amino groups [26–28]. Increasing ionic strength and/or pH decreases binding affinities. No significant change in affinities was

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