

A-site model RNAs

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Abstract

Oligonucleotide model systems have played a key role for the exploration of structure, dynamics and ligand binding of the ribosomal decoding-site (A site). An overview is given on the merits and limitations of various A-site models that were used for solution and crystallographic work.

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1. Introduction

The A site within bacterial 16S rRNA in the 30S subunit provides the decoding mechanism by which the ribosomal machinery deciphers the mRNA code. Matching of the mRNA codon and the anticodon of the aminoacylated tRNA is monitored by direct interaction with adenine bases of the decoding-site region, which at its core consists of a small internal loop within rRNA (Fig. 1). Biochemical and structural analyses revealed that the unpaired adenine bases 1492 and 1493 are conformationally flexible [1]. During the decoding process, transitions occur between distinct conformational states of these residues, at one extreme projecting both bases away from the RNA helix where they contact directly the codon–anticodon hybrid. Besides its decoding function during translation, the A-site loop has been identified as the target for natural aminoglycoside antibiotics [2] (Fig. 2) which kill bacteria by reducing decoding fidelity of the ribosome. The mechanism of action of the aminoglycosides has been linked to their ability to bind specifically to the bacterial A-site loop and thereby lock the conformation of adenines 1492 and 1493 in a state that is productive for peptide bond formation for both cognate and near-cognate tRNA–mRNA complexes [3,4].

Both the properties of the A site as an RNA conformational sensor and as the target of natural antibiotics have spurred research efforts on this ribosomal domain. Many significant

studies of the A site have been performed on model systems. The A-site motif stands out among ribosomal RNA targets in that it can be isolated from the ribosomal context and inserted into model oligonucleotides that retain the dynamic and aminoglycoside binding characteristics. Here, I summarize the various A-site models and discuss their merits and limitations for the study of static, dynamic, structural and ligand binding properties of the ribosomal target.

2. A-site models for solution studies

Chemical footprinting investigation of 16S rRNA during ribosomal subunit assembly led to the suggestion that small RNA domains that are devoid of extensive protein interactions might fold and function autonomously [5]. Purohit and Stern were the first to demonstrate that the decoding-site region of 16S rRNA could be isolated from the context of the whole ribosome and inserted into a small oligonucleotide, retaining the aminoglycoside binding properties of the ribosomal site [6]. They used an RNA construct that contained the bacterial A-site sequence flanked by a series of stabilizing base pairs (“clamping helix”) and a UUCG tetraloop (Fig. 1, construct I). Chemical footprinting of this model RNA in the presence of decoding-site binding aminoglycosides such as neomycin and paromomycin (Fig. 2) resulted in nucleotide protection pattern closely matching those obtained with whole ribosomes. Interestingly, hygromycin B, which binds at a region just above the A site, also elicited footprinting changes at the model RNA, consistent with the fact that the binding site of this aminogly-

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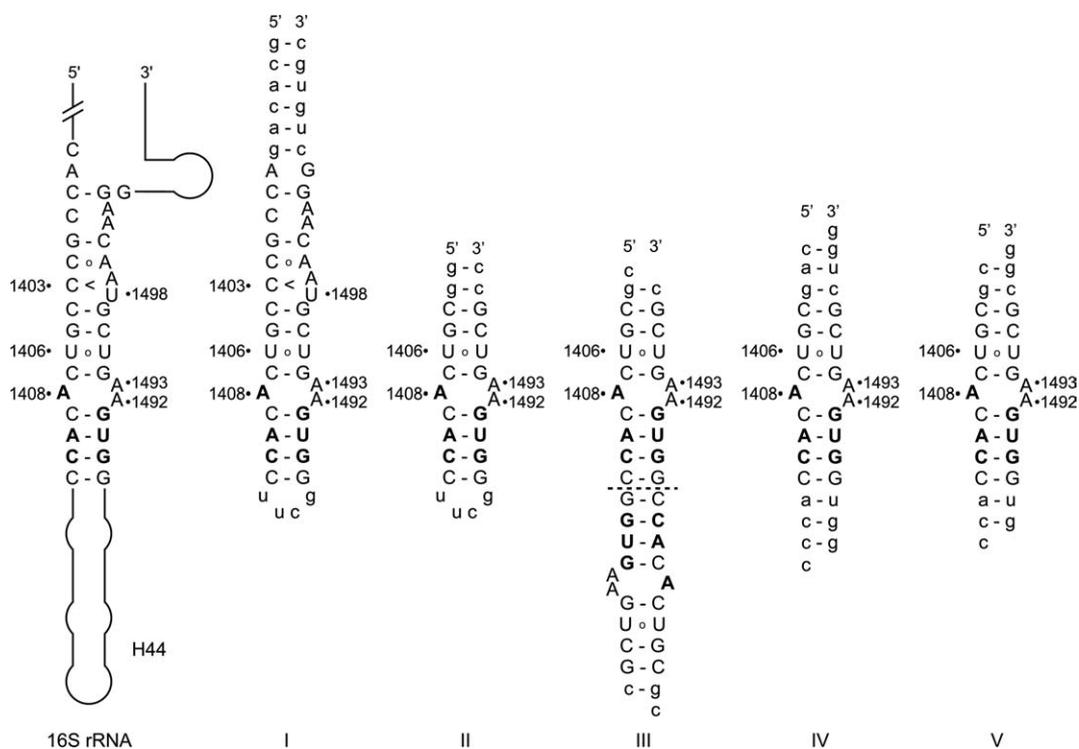


Fig. 1. Oligonucleotide models of the ribosomal decoding-site (A site) RNA. The bacterial A site is a small internal loop of three unpaired adenines (1408, 1492, and 1493), flanked by a non-Watson–Crick U \circ U pair (1406 and 1495), within helix 44 of 16S rRNA. Residues that are specific to the bacterial sequence are shown in bold. Nucleotide numbering is according to the *E. coli* 16S rRNA sequence. Hairpin models I (Purohit/Stern [6]) and II (Puglisi/Miyaguchi [8,9]) consist of a single strand that folds into the decoding-site sequence capped by a tetraloop. Models III (Vicens/Westhof [35]), IV and V (both Hermann [45,46]) are formed by hybridization of two single strands. The bipartite model III contains two identical copies of a self-complementary strand. Model III has been used with the terminally overhanging C (as shown), or with two uridines instead of the C.

coside around the C1403 < (A1499/U1498) triple was part of the Purohit/Stern construct (Fig. 1).

The finding that a small model oligonucleotide could be used to study aminoglycoside binding to the bacterial A site was seminal to a large number of following efforts to characterize ligand interaction with this ribosomal target. In the absence of the bulk of other ribosomal components that render experimentation and interpretation of results difficult, models of the A site proved advantageous for studying in detail structural and dynamical aspects of ligand binding to RNA. Importantly, model A-site oligonucleotides allowed, for the first time, to obtain three-dimensional structure information for a small-molecule complex of rRNA. Puglisi and coworkers performed NMR structure determination on the paromomycin complex of a minimal A-site model (Fig. 1, II) [7] that was derived from the Purohit/Stern construct by removing upper parts of the hygromycin binding region and shortening the “clamping helix” while retaining the closing UUCG tetraloop [8]. Concurrently with this work, Miyaguchi et al. [9] suggested and validated the same minimal A-site construct by chemical footprinting. The NMR structural studies were accompanied by biochemical investigations of the minimal A-site model that proved its usefulness as a faithful mimic of the ribosomal decoding domain [8,10–13]. Specifically, the model was key to studying species-specific sequence determinants for aminoglycoside binding to rRNA [8], the impact of resistance mutations [10], and conformational changes induced in the

RNA by aminoglycoside binding [13]. The resulting data supported the notion that the artificial sequence parts, specifically the tetraloop, which were added to stabilize the A-site loop in a small oligonucleotide construct did not interfere with selective ligand binding to the A-site target.

In addition to the paromomycin complex the Puglisi/Miyaguchi oligonucleotide construct was also used to obtain NMR structures for gentamicin bound to the bacterial A site [14] and paromomycin bound to the eukaryotic sequence [15]. While some binding studies of aminoglycosides were performed with longer oligonucleotide models that resembled the earlier Purohit/Stern construct [16–18], the Puglisi/Miyaguchi minimal A-site construct was quickly adopted by other groups. Wong and coworkers used surface plasmon resonance to study aminoglycoside interactions with the minimal A-site construct [19,20]. Binding of fluorescently-labeled aminoglycosides to the Puglisi/Miyaguchi oligonucleotide was investigated by Ryu and Rando [21,22]. A-site interactions of aminoglycosides modified by bacterial resistance enzymes were studied by Chow, Mobashery and colleagues who described an affinity assay based on a 5'-fluorescein-labeled Puglisi/Miyaguchi construct [23,24]. The same RNA, 3'-modified with a non-fluorescent acceptor dye, was used for the development of a FRET assay that measured ligand binding as a function of the displacement of a fluorescently-labeled aminoglycoside surrogate. Combination of the FRET assay with an in silico docking approach yielded several novel non-

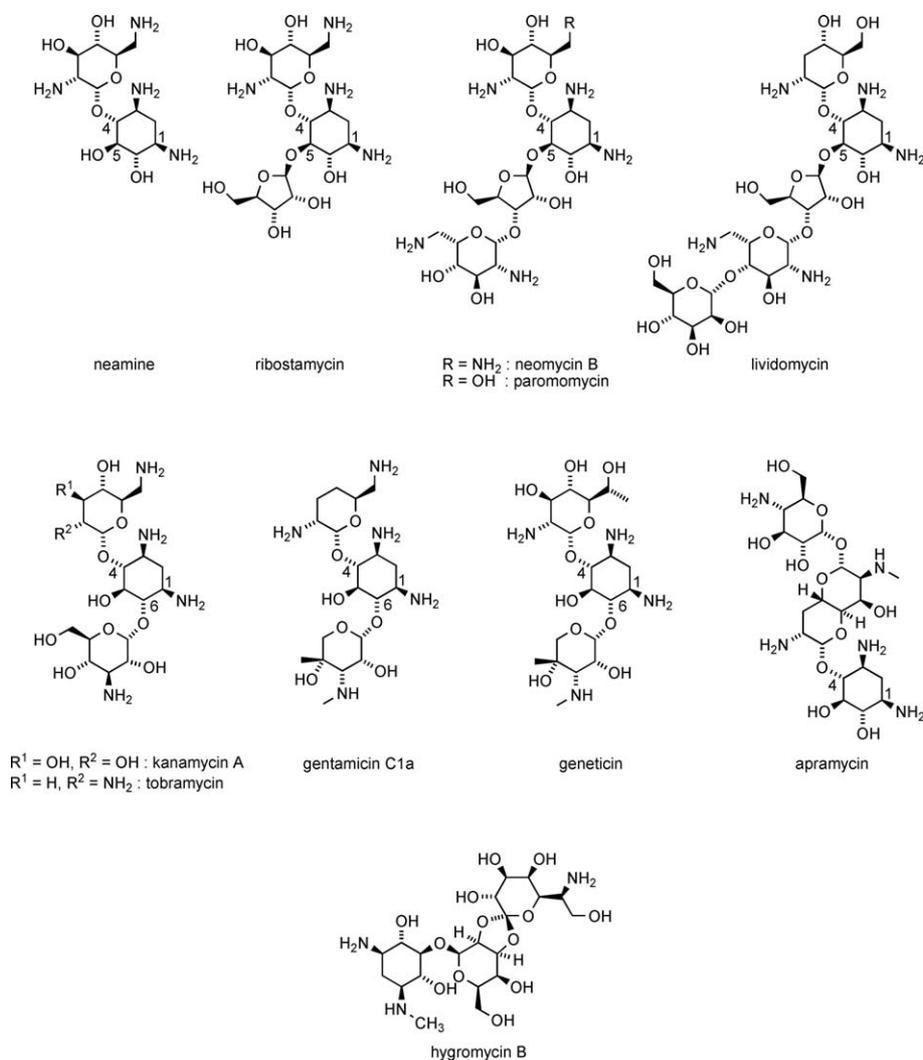


Fig. 2. Aminoglycoside antibiotics that target the bacterial A site and hygromycin B, a ligand that binds to helix 44 of 16S rRNA in proximity of the A site. See text for details.

aminoglycoside ligands of the bacterial A site [25]. High-throughput screening of small-molecule libraries against the A-site target was also the goal of a mass-spectroscopy-based assay developed at ISIS Pharmaceuticals that made use of the Puglisi/Miyaguchi oligonucleotide [26–28]. Satisfyingly, many of the observations of aminoglycoside binding to the minimal A-site construct obtained by various solution techniques were consistent with data collected with the same oligonucleotide by the gas-phase MS technique. Application of the method to sequence variants, including the eukaryotic A site, revealed determinants of aminoglycoside binding specificity for the bacterial rRNA target [27]. Finally, the Puglisi/Miyaguchi A-site construct has been extensively used in calorimetry experiments by Pilch and coworkers who studied the thermodynamics of aminoglycoside recognition [29–33].

3. A-site models for crystallographic studies

In essence, the oligonucleotide developed by the Puglisi and Miyaguchi groups proved to be an extremely useful model to study structure and ligand interaction of the A-site RNA unob-

structed by the bulk of other ribosomal components. The tetraloop-capped construct provided a faithful mimic of the A-site domain, attested by the consistency of data obtained with a variety of distinct experimental techniques. While aiding solution studies on the A site by avoiding detrimental influences of excess single strand RNA, the tetraloop hindered crystallographic work. Previous attempts to crystallize short RNA hairpin loops were frustrated by the propensity of such constructs to crystallize as dimers of hybridized strands in which the loop sequence is accommodated as an internal bulge (for a collection of references see [34]). Crystallographic work on the A site has thus focused on bipartite double-stranded constructs (Fig. 1, III–V) which usually contained terminally overhanging nucleotides that facilitate crystal packing.

An elegant model RNA for crystallography has been devised by Vicens and Westhof [35] who incorporated two antiparallel A-site motifs into a bipartite construct that was formed from two copies of a self-complementary oligonucleotide (Fig. 1, III). As this A-site model does not require reconstitution from two different strands, the Vicens/Westhof RNA combines the advantage of a monopartite hairpin construct

with a design that is compatible with crystallization. As an added benefit, the presence of two binding sites potentially allows the comparison of slight conformational distinctions within a single crystal structure.

The Vicens/Westhof model has provided three-dimensional structure information for over 10 A site-bound aminoglycosides so far [36,37], including the complexes of paromomycin [35], a paromomycin derivative [38], tobramycin [39], geneticin [40], gentamicin C1A [41], neomycin B [41], lividomycin [41], ribostamycin [41], kanamycin A [41], neamine [41], a semi-synthetic neamine derivative [42], and apramycin [43]. Except for neamine and a neamine derivative, these complexes showed ligand occupation of both A sites in the construct albeit one of the sites was usually better defined than the second. In the geneticin and apramycin structures both A sites were well resolved, revealing alternative conformations for the flipped-out adenine residues 1492 and 1493 for the two sites. The crystal structures of ribostamycin and kanamycin showed secondary binding sites of the aminoglycoside located in close proximity of the A site at the entrance of the major groove. While these non-specific binding sites might be considered idiosyncrasies of the particular RNA construct, it has been suggested that they might be exploited to design dimeric aminoglycosides comprised of the A site-bound and the non-specific ligand [41]. Interestingly, in the complexes of neamine and a derivative thereof, the signature U1406 \circ U1495 base pair (Fig. 1) was disrupted with U1406 bulging out from the duplex and U1495 forming an intramolecular base pair with A1492 of a neighboring RNA in the crystal lattice [41,42].

Comparison of the three-dimensional structures of aminoglycoside complexes obtained by NMR for the Puglisi/Miyaguchi construct [7] and crystal structures solved for the Vicens/Westhof RNA as well as for whole 30S ribosomal subunits confirmed the validity of the A-site models [36,37,44]. Structural features of the RNA around the aminoglycoside binding region were virtually identical in the paromomycin cocrystal structures of the 30S subunit [3,4] and in the Vicens/Westhof construct [35]. Specifically, the conformations and positions of the bulged-out residues A1492 and A1493 were very similar in both crystal structures despite the fact that, in the model RNA, these adenines were involved in crystal packing contacts. While the binding position of the aminoglycoside core was comparable between the crystal and solution structures, the residues A1492 and A1493 showed distinct conformations in the NMR model. The impact of the dynamic nature of the A-site motif on conformational differences observed between solution and crystallographic methods has been discussed extensively [35,36,44].

To obtain an A-site model that would be equally suited for crystallographic and solution studies, Hermann and coworkers designed bipartite RNA constructs that included the internal loop flanked by two stabilizing helix regions (Fig. 1, IV and V) [45,46]. These model systems emerged from crystallization screening of oligonucleotides with varying lengths of flanking stems and terminal overhangs. Notwithstanding the seemingly simple design of the constructs, previous efforts to obtain bipartite A-site oligonucleotides for crystallization were un-

successful as they yielded artifactual homodimers containing only one of the models' strands [34]. The constructs IV and V were used to determine crystal structures of the free A site and of complexes with neomycin and a conformationally restricted neomycin derivative [45]. Solution studies of the Hermann constructs labeled with the fluorescent base 2-aminopurine at the positions 1492 or 1493 further confirmed the dynamic nature of the A site [46]. In contrast to the Vicens/Westhof construct, only A1493 was involved in packing contacts to a neighboring RNA molecule while A1492 remained flexible. Thus, model IV allowed the capture of two conformational states of A1492 within a single crystal. This residue was either oriented inside the RNA helix and forming a base pair with A1408, or bulged-out from the RNA and stacking on A1493. By variation of the crystallization conditions occupancy of either state could be driven to completeness. Crystallographic and fluorescence investigations of the Hermann constructs along with crystal structures of the Vicens/Westhof model system and the 30S subunit clearly demonstrated that the free A site exists in multiple alternative conformations while aminoglycoside binding locks A1492 and A1493 in a single bulged-out state [41]. The fluorescently-labeled constructs IV and V were also the basis for a binding assay that was used to test A-site ligands as lead molecules for the discovery of novel antibiotics [46–48].

4. Future perspective

Oligonucleotides containing the A site have been extraordinarily useful to study the structure, dynamics, and molecular recognition of this important ribosomal target. Even in the presence of structure information for the whole 30S subunit [1], and for the whole ribosome [49], small RNA model systems will remain to be crucial for the elucidation of structural details of ribosomal targets at high resolution. This is especially true for the study of ribosomal targets for antibacterial ligands [50–52]. While the majority of known antibiotic binding sites in the ribosome are comprised of motifs that are separated within the rRNA sequence, and as such are not easily amenable to a model system approach, new potential targets have been described [53] that might be studied using smaller RNA constructs.

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