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Decoding Region Bubble Size and Aminoglycoside Antibiotic Binding

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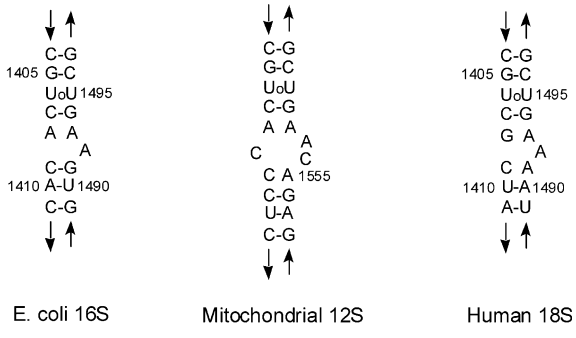
Abstract—Aminoglycoside antibiotics promiscuously bind to structurally diverse RNA molecules containing internal bubbles and bulges with affinities in the μM range. An interesting exception is found in the human 12S mitochondrial decoding region where aminoglycoside binding, unlike in the case of its bacterial and human cytoplasmic counterparts, is absent. Mutations that reduce the size of the bubble in the 12S decoding region immediately restore aminoglycoside binding, giving the system chemical switch like behavior.

Molecular studies on the specificity and structural basis of RNA–drug interactions represents an emerging area in chemical biology. An essential issue in this area relates to the question of whether the flexibility of RNA molecules is such as to allow for the design of highly specific antagonists directed against a particular RNA molecule. Studies on the aminoglycoside antibiotics are exceedingly instructive in this regard.

Aminoglycosides function as antibiotics by binding to the A-site rRNA decoding regions of prokaryotes.[1–3] This binding results in either mistranslation or premature termination of protein biosynthesis.[1–3] The binding of aminoglycosides to sensitive ribosomes[4] and decoding region constructs[5,6] occurs in the μM range. Affinities in this range often do not lead to highly specific binding interactions. While the binding of aminoglycosides to prokaryotic A-site RNA constructs shows some specificity, as judged by NMR structural studies,[7] binding studies with structurally diverse aminoglycosides do not suggest a high order of specificity with respect to aminoglycoside structure.[6]

Moreover, binding affinities of aminoglycosides to prokaryotic decoding region constructs also are not very sensitive to mutations within the RNA binding region.[6] Binding studies also demonstrate that aminoglycosides can bind to eukaryotic decoding region constructs with approximately the same affinities found with their prokaryotic counterpart, even though the two structures are different (Scheme 1).[6]

In fact, aminoglycosides can bind in a saturable fashion to a wide variety of RNA structures with similar μM affinities. For example, aminoglycosides have been found to bind to HIV RRE and TAR RNA regions,[8,9] thymidylate synthase RNA[10] as well as to various RNA aptamers selected against aminoglycosides.[11–13] All of these RNA molecules contain non-duplex structural elements, generally either bulges or bubbles.[14] Presumably the A-site major groove is too narrow to sterically allow access of the bulky aminoglycosides to the purine and pyrimidine bases.[15] Hydrogen and electrostatic



Scheme 1. Prokaryotic and eukaryotic A-site decoding regions.

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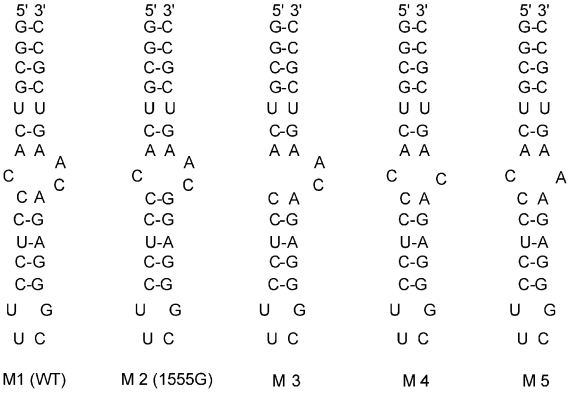
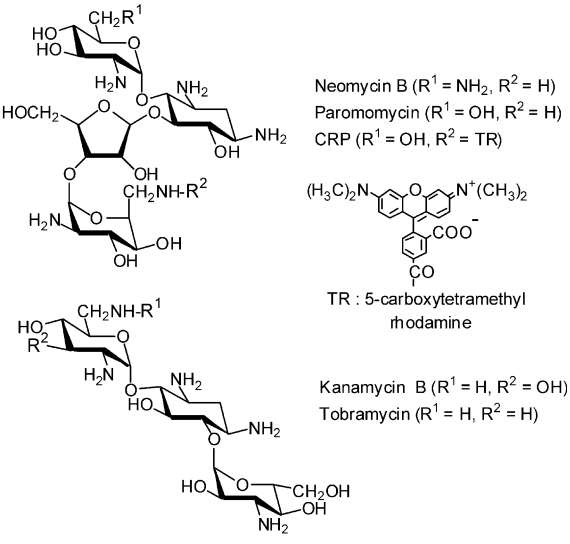
bonding between the aminoglycosides and the bases appears to facilitate binding,[7] although weak electrostatic interactions with the phosphate backbone of RNA are also possible.[16]

Given the structurally diverse group of non-A form RNA structures that specifically bind aminoglycosides, an essential issue to address is whether essentially all non-A form duplex structures bind aminoglycosides, and what are the structural features important for binding. The 12S mitochondrial rRNA decoding region is of interest along these lines (Scheme 1). A point mutation (1555^{G}) in the human mitochondrial 12S decoding region rRNA leads to severe aminoglycoside-induced ototoxicity.[17] Large (110 nts) rRNA constructs of WT and the 1555^{G} mutant demonstrate that while the mutant can bind aminoglycosides with approximately the same affinity as does the 16S bacterial decoding region construct (Scheme 1), the WT 12S rRNA construct does not bind aminoglycosides.[18] This is a surprising result, and shows that a point mutation can lead to substantial differences in binding affinities for aminoglycosides, and in this case aminoglycoside-induced deafness. An important issue to address is the nature of the structural change that accompanies the transition from aminoglycoside binding to non-binding.



Scheme 2. Mitochondrial 12S rRNA constructs.



Scheme 3. Structures of aminoglycosides used in studies.

Simple secondary Mfold[19] structural analysis suggests that the 1555^{G} point mutation leads to the formation of a new base pair, thus reducing the size of the internal bubble (Scheme 2).[18] This suggests then that there may be a relationship between internal bubble size and the ability to bind aminoglycosides. This idea is directly tested here, and it is shown that there is a direct relationship between aminoglycoside binding and bubble size in the 12S rRNA series.

The obvious difference between the three decoding regions shown in Scheme 1 is that the 12S decoding region possesses a larger internal bubble than its 16S and 18S counterparts. That bubble size might be correlated to binding activity is suggested by the 1555 (G/A) mutation, which causes enhanced aminoglycoside induced ototoxicity.[17] As mentioned above, a 110 nt 12S construct is unable to specifically bind aminoglycosides, while the 1555G mutant specifically binds aminoglycosides.[18] In the studies described here, truncated 12S rRNA constructs were prepared to test the hypothesis that aminoglycoside binding is related to bubble size. The constructs prepared are shown in Scheme 2. Binding of aminoglycosides to these constructs was determined

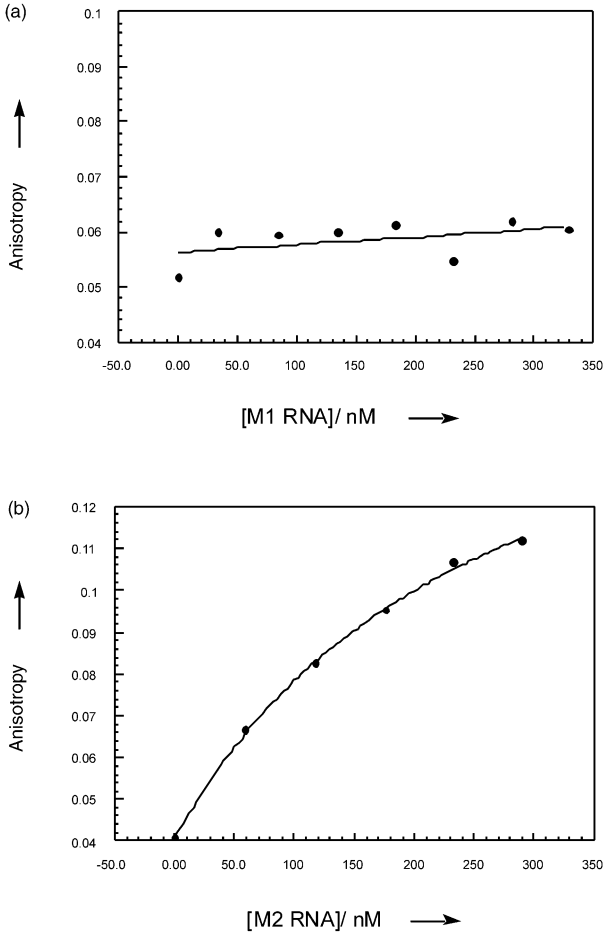


Figure 1. Fluorescence anisotropy of fluorescently labeled paromomycin (CRP) (20 nM) as a function of 12S rRNA construct, M1 (A) and M2 (B) concentrations. In these experiments, increasing concentrations of the RNA constructs were added to 20 nM CRP under the conditions described in refs 5 and 6.

Table 1. Dissociation constants for binding of aminoglycosides to mitochodrial 12S rRNA constructs [K_d (binding to CRP), K_D (binding to aminoglycosides), μM]

RNA	CRP (K_d)	Neomycin	Paromomycin	Kanamycin	Tobramycin
M1(WT)	NB[a]				
M2	0.27±0.035	0.14±0.021	3.83±0.21	3.46±0.21	3.26±0.47
M3	0.26±0.049	0.12±0.012	0.44±0.071	2.08±0.25	0.43±0.085
M4	0.75±0.014	0.16±0.014	12.6±1.98	13.6±0.85	2.91±0.19
M5	0.33±0.049	3.97±0.026	5.85±0.76	2.64±0.20	5.37±0.39

[a]No binding.

by fluorescence anisotropy measurements according to previously published methods.[5,6] A fluorescently labeled paromomycin derivative (CRP) (Scheme 3) is used to directly measure binding.[5,6] Competition experiments between CRP and the aminoglycosides shown in Scheme 3 provide dissociation binding constants for the interactions of the drugs to the RNA constructs. Protocols for these binding studies have been previously published.[5,6] Figure 1 shows the binding isotherms for CRP to the wild-type 12S (M1) construct and its 1555G mutant (M2). This figure demonstrates that the wild-type 12S construct is unable to bind CRP, and that the 1555G mutation restores binding affinity in these truncated constructs (Table 1). Thus, the truncated RNA constructs behave just as the 110 nt fragments alluded to above.

To test the idea that aminoglycoside binding is related to bubble size, the further constructs shown in Scheme 2 were prepared and tested for aminoglycoside binding affinities. The three constructs (M3–M5) have one different nucleotide removed from the bubble of the wild-type construct M1. In all cases μM binding affinities for aminoglycosides were restored, although individual differences in dissociation constants for the binding of aminoglycosides were manifest (Table 1). It is noteworthy that M3 exhibits the highest affinity binding. It should be mentioned that the observed binding affinities are rather similar to those measured with bacterial 16S decoding region constructs.[6] For example, the measured K_D values in the case of the bacterial construct for neomycin, paromomycin, kanamycin, and tobramycin are 0.053, 1.65, 1.25, and 1.40 μM, respectively.[6]

The results reported here are entirely consistent with the hypothesis that bubble size in the 12S series is strongly related to the capacity of the constructs to bind aminoglycosides and that simply removing one nucleotide from the bubble restores binding. The fact that aminoglycosides can bind in a saturable fashion to so many structurally diverse RNA structures in the μM range renders these results somewhat surprising. This can be readily seen in the interactions of aminoglycosides with HIV–RRE RNA constructs.[14] Binding of aminoglycosides occurs at an internal bubble region and the gradual conversion of the bubble region into an A-form duplex only gradually reduces affinities for aminoglycosides as long as non-duplex elements are still present.[14] What is most surprising about the 12S constructs is that there is an abrupt change from high affinity aminoglycoside binding to no binding in one step. That the wild-type 12S decoding region cannot bind aminoglycosides is of essential importance for the clinical use of these drugs.[17] While the structural parameters underlying the inability of wild-type 12S constructs to bind aminoglycosides is unknown, the results described here would suggest that the size of the bubble is of central importance. One possible reason for this is the presumed greater flexibility possible with larger bubbles. A situation akin to this may be seen in the lack of binding of Rev peptides to RRE RNA and an RRE mutant (M4) which possesses an expanded internal bubble region.[20] Limits to the degrees of freedom in the flexibility of the large internal bubble to accommodate ligand binding may be prohibitively costly from an energetic standpoint. Interestingly, the simple step function from an aminoglycoside binding to a non-binding state in the 12S constructs has the makings of a chemical switch.

Acknowledgements

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