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Which Aminoglycoside Ring is Most Important for Binding? A Hydropathic Analysis of Gentamicin, Paromomycin, and Analogues

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Abstract—The NMR structures of gentamicin and paromomycin in complex with the A-site of *Escherichia coli* 16S ribosomal RNA were modified with molecular modeling to 12 analogues. The intermolecular interactions between these molecules and RNA were examined using the HINT (Hydropathic INTeractions) computational model to obtain interaction scores that have been shown previously to be related to free energy. The calculations correlated well with experimental binding data, and the interaction scores were used to analyze the specific structural features of each aminoglycoside that contribute to the overall binding with the 16S rRNA. Our calculations indicate that, while ring I binds to the main binding pocket of the rRNA A-site, ring IV of paromomycin-based aminoglycosides contributes significantly to the overall binding.

Aminoglycoside antibiotics have been used for five decades in the treatment of tuberculosis, pneumonia, and other gram-negative bacteria.[1] Although the precise mechanism of action is unknown, aminoglycosides are known to bind with the 16S ribosomal RNA A-site, which is involved in the interaction between the codon and the aminoacyl-tRNA anticodon. This interaction is involved in maintaining the fidelity of the genetic code, so the result of interfering with it is a disruption in protein synthesis by inducing codon misreading.[2] Issues of toxicity, bacterial resistance, and limited oral bioavailability have stimulated further drug design and development.[3] NMR data indicates that rings I and II of the paromomycin-based analogues lie in the A-site pocket of rRNA, formed by the bulged nucleotide A^{1492} and the A^{1408}–A^{1493} base pair.[4] Little emphasis has been placed on rings III and IV, as they are described as contributing weakly to specific binding and function. The purpose of our experiment is to further evaluate the interactions between the A-site of 30S ribosomal RNA with the different rings of a variety of paromomycin and gentamicin-based aminoglycoside analogues (Fig. 1) using molecular modeling and computational chemistry techniques.

Molecular models were created and minimized using the Tripos force field and Gasteiger–Hückel charges with the SYBYL 6.6 molecular modeling package.[5] The starting points were averaged NMR structures of the 16S rRNA A-site of paromomycin[6] (1pbr), a 4,5-linked 2-deoxystreptamine derivative, and gentamicin[7] (1byj), a 4,6-linked 2-deoxystreptamine derivative, obtained from the PDB. Specific amines in the models were protonated as previously described.[8,9] Since the core structures of these two classes of aminoglycosides are similar, 12 analogues were created by simple modification of the atoms directly in SYBYL. A 6 Å sphere was defined surrounding the aminoglycoside molecule, and all portions of the structure outside of this sphere were defined as an aggregate to maintain the integrity of the RNA. All complexes were then treated with 300 cycles of steepest descent energy minimization followed by conjugate gradient minimization until the energy difference was less than 0.05 kcal mol^{-1}. Also, we found it necessary to use a distance-dependent dielectric constant of 8 to temper an unreasonably strong attraction between the highly negative phosphate oxygen atoms in the RNA and ammonium ions of the aminoglycosides.

The interactions of each complex were then analyzed using the HINT (Hydropathic INTeractions) program.[10–13] The HINT model describes specific interactions between two molecules, RNA and the aminoglycoside in our case, as a double sum over the atoms within each component (eq 1).

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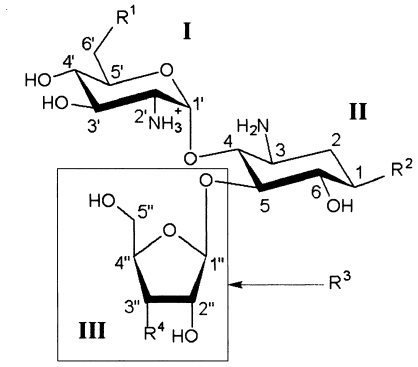
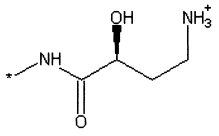
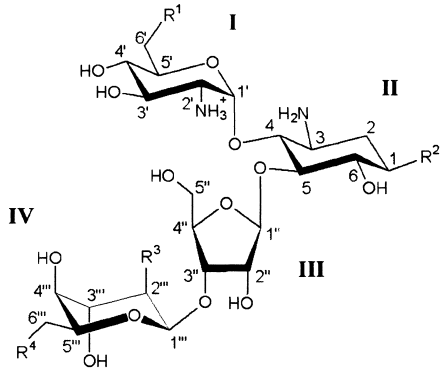


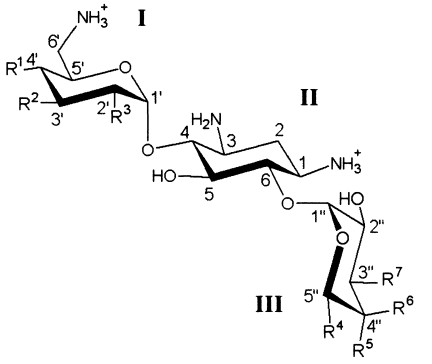
1. $R^1 = -NH_3^+$, $R^2 = -NH_3^+$, $R^4 = -OH$
2. **1**, $R^2 =$



3. $R^1 = -NH_3^+$, $R^2 = -NH_3^+$, $R^3 = -OH$
4. **3**, $R^1 = -OH$
7. $R^1 = -NH_3^+$, $R^2 = -NH_3^+$
   $R^4 = -(CH_2)_2NH_3^+$
8. **7**, $R^4 = -(CH_2)_2NH(CH_2)_3NH_3^+$



5. $R^1 = -OH$, $R^2 = -NH_3^+$, $R^3 = -NH_3^+$, $R^4 = -NH_3^+$
6. **5**, $R^1 = -NH_3^+$
9. **6**, $R^3 = -OH$, $R^4 = -OH$
10. **6**, $R^3 = -OH$, $R^4 = -NH_3^+$



11. $R^1 = -H$, $R^2 = -H$, $R^3 = -NH_3^+$, $R^4 = -H$,
    $R^5 = -CH_3$, $R^6 = -OH$, $R^7 = -NHCH_3$
12. $R^1 = -OH$, $R^2 = -OH$, $R^3 = -OH$, $R^4 = -CH_2OH$,
    $R^5 = -OH$, $R^6 = -H$, $R^7 = -NH_3^+$
13. **12**, $R^3 = -NH_3^+$
14. **12**, $R^2 = -H$, $R^3 = -NH_3^+$

Figure 1. Chemical structures of the aminoglycoside antibiotics.

$$B = \sum_{j=1}^{\text{atoms}} \sum_{i=1} b_{ij} = \sum \sum \left(a_i S_i a_j S_j R_{ij} T_{ij} + r_{ij} \right) \quad (1)$$

a is the hydrophobic atom constant derived from $\text{Log}P_{o/w}$, S is the solvent accessible surface area, T is a function that differentiates polar–polar interactions (acid–acid, acid–base or base–base), and R, r are functions of the distance between atoms i and j. The binding score, b_{ij}, describes the specific interaction between atoms i and j, and B describes the total interaction.

This study was performed with the 2.35S version of HINT, as reported previously.[12] The HINT parameters for the aminoglycoside analogues were calculated using a partitioning algorithm based on the CLOGP method of Hansch and Leo,[14] and the RNA oligonucleotides were assigned HINT parameters using the HINT dictionary.[12] Only polar hydrogens were explicitly used in the model. Equation 1 was used to calculate an interaction score for each structurally minimized aminoglycoside/rRNA complex. Interaction scores were further calculated between the rRNA and the individual rings I–IV of all compounds to gain insight into the total interaction contribution of each ring (Table 1). HINT 3-D interaction maps showing the type and quality of noncovalent interactions between 16S rRNA and paromomycin (Fig. 2a) and gentamicin C_{1a} (Fig. 2b) were calculated.

Ring I of paromomycin (compound **5**) is positioned within a pocket created by the bulged nucleotide A^{1492} and the A^{1408}–A^{1493} base pair,[4] and there are hydrophobic interactions created by stacking of this ring against the G^{1491} base pair (Fig. 2a). It appears, however, that the polar interactions of the N2′ ammonium as well as the three hydroxyl oxygens contribute more to the positioning of ring I within this pocket. A strong hydrogen bond (2.69 Å) is observed between the N2′ ammonium and the phosphate oxygens of G^{1491}. There is also a good hydrogen bond between the phosphate oxygen of A^{1492} and the 3′-OH of the aminoglycoside with a contact distance of 2.78 Å. There is also evidence to support a hydrogen bond[15] (3.1–3.4 Å) between the 6′-OH and the carbon–nitrogen π bond in A^{1492}. Replacement of this 6′-OH with an ammonium to form neomycin B (compound **6**) indicates this interaction as well (3.1–3.6 Å), in addition to a hydrogen bond (2.8–3.3 Å) with the carbon–nitrogen π bond in A^{1493}. This information, combined with a strong polar interaction between the N6′ ammonium and the phosphate oxygen of A^{1493}, explains the higher overall HINT score of neomycin B (**6**) versus paromomycin, as well as the higher individual HINT scores of ring I of all five neomycin B analogues versus ring I of paromomycin (Table 1).

Ring II of paromomycin is positioned between the U^{1406}–U^{1495} bases and the C^{1407}–G^{1494} base pair, and is connected to ring I via a flexible ether oxygen linkage. The HINT score data for ring II indicates that this ring and its substituents contribute to the binding with rRNA less than half as much, on average, as ring I. Nevertheless, there are still significant interactions with the N1 ammonium, the N3 amino group, and the O6

hydroxyl. The N1 ammonium forms a strong hydrogen bond (2.84 Å) with a carbonyl oxygen in U^{1495} and the N3 amino group forms a hydrogen bond (3.11 Å) with a phosphate oxygen of A^{1493}. These two amino groups are present in one form or another, in every aminoglycoside in this study, and are believed to be required for specific binding of the drug to rRNA.[4] Lastly, the 6-OH appears to have a weak acid–base interaction with a carbonyl oxygen of U^{1406} at a distance of 4.3 Å, which could, nonetheless, have potential influence on the positioning of rings III and IV.

Compound **2** contains a modification at position 1 which moves the positively charged ammonium four carbons away from the ring. This significantly enhances the overall contribution of ring II to binding by allowing a strong hydrogen bond (2.66 Å) to form with the phosphate oxygen of U^{1495}. The overall HINT score and ΔG of this compound is still less than that of neomycin B and paromomycin due to the absence of ring IV.

There do not appear to be many significant interactions between the five-membered ring III and the rRNA, and the HINT scores for this ring indicate minimal, if any, contributions to binding for all of the 4,5-linked analogues. However, a hydrogen bond is made between the 5″-OH and a phosphate oxygen of G^{1491}, which could aid in the orientation and positioning of ring IV. There also appears to be an internal hydrogen bond between the 2′-NH_3^+ of ring I and the 4″ oxygen atom of ring III, contributing to the distinctive 'L'-shape of these compounds.[4] Therefore, it would seem that this ring mostly serves as a linker between rings I and II and ring IV.

There is good evidence of significant contributions of the six-membered ring IV of paromomycin to the binding of aminoglycoside with rRNA (Table 1). The N2‴ ammonium participates in hydrogen bonding interactions with the phosphate oxygens of U^{1406} (3.97 Å), G^{1489} (3.42 Å) and U^{1490} (2.69 Å). The O3‴ and O4‴ hydroxyl groups hydrogen bond with the phosphate oxygens of C^{1407} (3.25 Å) and G^{1489} (2.72 Å), respectively. The N6‴ ammonium also forms two strong hydrogen bonds with the carbonyl oxygens of U^{1490} (2.79 Å) and G^{1491} (3.54 Å) (Fig. 2a). The contributions of ring IV can further be explored by several analogues of neomycin B. While compound **6** is identical to paromomycin with respect to ring IV, and interacts in a similar manner to paromomycin, compounds **7** to **10** contain significant variations to the structure of 'ring

Table 1. Thermodynamic binding data, total HINT score and HINT score by ring for aminoglycoside antibiotics bound to the ribosomal RNA A-site

Compound	K_D[16,17] (μM)	ΔG (kcal mol^{-1})[a]	HINT interaction units				
			Total	Ring I	Ring II	Ring III	Ring IV
1 Ribostamycin	25	−6.27	6486	4298	1587	308	
2 Butirosin B	27	−6.23	7537	3707	3487	−94	
3 Neamine	7.8	−6.96	7364	4883	2302		
4 Paromamine	< 100	−5.45	6766	4666	1902		
5 Paromomycin	0.2	−9.13	12,334	3584	1726	215	6162
6 Neomycin B	0.019	−10.52	13,455	4743	1724	194	6167
7 Analogue W	1.7	−7.86	10,052	4432	1638	−20	3698
8 Analogue X	0.26	−8.98	9925	4596	1664	204	2834
9 Analogue Y	28	−6.21	8724	4769	1707	228	1372
10 Analogue Z	0.7	−8.39	9816	4768	1718	225	2495
11 Gentamicin	1.7	−7.86	4537	1488	2051	689	
12 Kanamycin A	18	−6.47	7388	2288	1771	3077	
13 Kanamycin B	1.4	−7.98	8273	3016	1863	3021	
14 Tobramycin	1.5	−7.94	7496	2295	1833	3031	

[a]Calculated ΔG values based on K_D binding data.

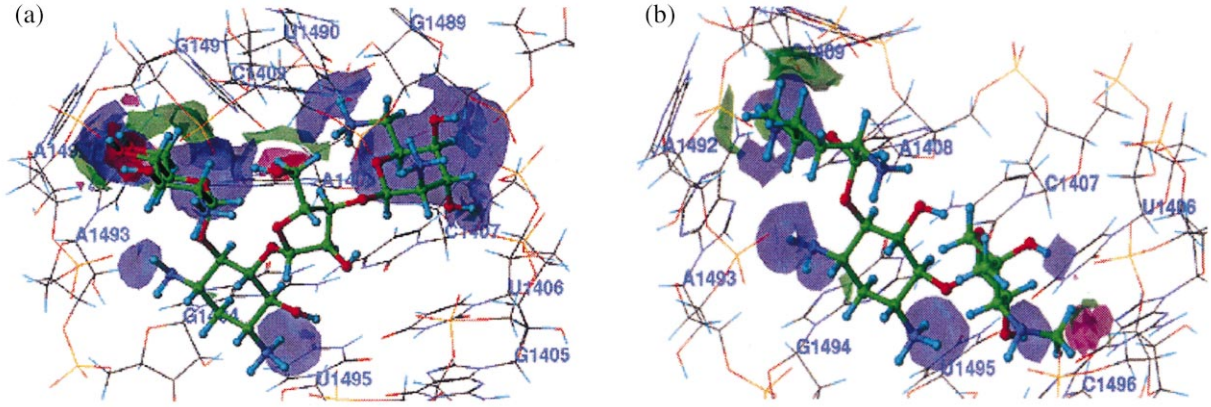


Figure 2. HINT interaction maps for (a) paromomycin and (b) gentamicin in the A-site of ribosomal RNA. A contour level of 115 was used. Blue areas represent regions of favorable polar interactions, red represents unfavorable polar interactions and green represents favorable hydrophobic interactions, magenta represents unfavorable hydrophobic interactions.

IV'. Compound **7** replaces the ring structure with a quaternary ethyl amine. While this terminal ammonium is allowed to interact with the phosphate oxygens of G^{1405}, U^{1406}, and U^{1490}, the lack of the other functional groups as well as the ring structure reduced the score for this section by almost half. The overall HINT score and ΔG (Table 1) were also reduced. Compound **8** extends the chain of **7** by an *n*-propylamine. While this ammonium does still form hydrogen bonds with phosphate oxygens at the end, the hydrogen bonding with the secondary amine is reduced from **7**, resulting in a decreased score. Further evidence of the importance of ring IV is observed in compound **1**, which lacks ring IV entirely and therefore has a significantly reduced overall HINT score and ΔG. Compounds **3** and **4** do not contain rings III and IV and show smaller scores and less negative ΔG as well (Table 1).

The 4,6-linked derivatives (compounds **11**–**14**) each contain only three rings, and the HINT interaction scores are notably less than the 4,5-linked compounds containing four rings. Furthermore, the contributions of ring I are also significantly reduced in comparison to the other compounds. This is most likely due to the presence of a hydroxyl or a hydrogen instead of a tertiary ammonium at the 6′ position, resulting in reduced hydrogen bonding with the π bonds in this pocket. Compound **11** also lacks both the 3′- and 4′-OH as well. Ring II is nearly identical with that of the 4,5-linked aminoglycosides, and the change in the position of ring III from 5 to 6 of ring II does not appear to significantly alter the contributions of this ring to binding. Ring III is a six-membered terminal ring in these compounds, instead of a five-membered linker ring as in the 4,5-linked compounds. As a terminal ring, there is evidence that the functional groups contribute more to binding than rings I and II. The 2″-OH of compound **11** makes a hydrogen bond (3.15–3.42 Å) with the carbonyl oxygen of G^{1405}, and the 4″-OH makes another hydrogen bond (3.05 Å) with the phosphate oxygen of the same base pair. The presence of a 4″- and 5″-OH in compounds **12**, **13** and **14** allow for hydrogen bonds with the phosphate oxygens of G^{1405} and U^{1406} (Fig. 2b).

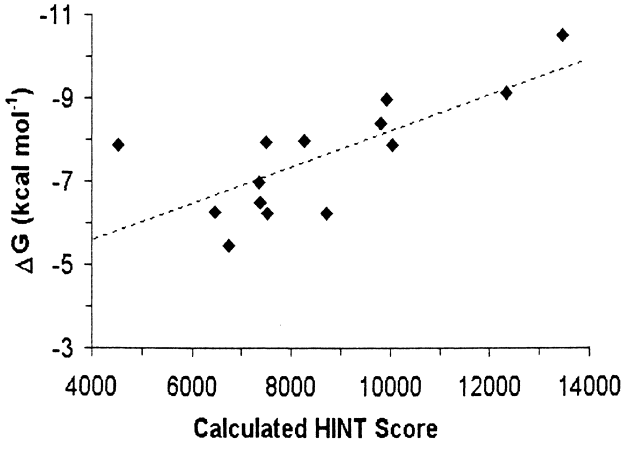


Figure 3. Calculated HINT interaction scores versus experimental ΔG.

Finally, to validate our calculations, the HINT scores were plotted against experimental ΔG values (Fig. 3). As we have seen previously,[11,13] HINT scores correlate with ΔG because $\mathrm{Log}P_{o/w}$ is a free energy measurement of solvent partitioning.[18] R for this correlation is 0.78. This result appears to have potential as a novel and predictive 3-D QSAR/pharmacophore model for similar aminoglycoside compounds bound to rRNA. The single outlier of Figure 3, gentamicin (**11**), may be due to the fact that a mixture of C_1, C_2, and C_{1a} gentamicin was used in measuring K_D whereas our calculations were performed on gentamicin C_{1a} (only).

In summary, our calculations indicate strong evidence that, while ring I is located in the main binding pocket of the A-site of rRNA, ring IV (and ring III of the 4,6-linked compounds) contributes significant polar interactions in the binding of rRNA to aminoglycosides. Future work is planned in using this information for the design of new aminoglycoside mimetics.

Acknowledgements

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