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Study of Aminoglycoside–Nucleic Acid Interactions by an HPLC Method

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Abstract—The interactions of a number of aminoglycoside antibiotics with tRNA and DNA were studied by an HPLC method, based on tRNA and DNA peak size exclusion. Among the compounds studied (deoxystreptamine, neamine, neomycin B, kanamycin A, gentamicin A, netilmicin, streptomycin, and the synthetic neamine analogue BKN3), neomycin B and the synthetic analogue of neamine were proved to be the most potent binders.

Aminoglycosides are a group of widely used broad-spectrum antibiotics with many desirable properties for the treatment of life-threatening infections.[1] They act primarily by impairing bacterial protein synthesis through binding to prokaryotic ribosomes. The neomycin-class aminoglycosides bind to a unique site on the ribosome causing a characteristic miscoding pattern common to all A-site binding antibiotics.[2] Streptomycin induces a different pattern of miscoding, allowing ribosomes to form initiation complexes and then prevent these complexes from continuing into the cycles of chain elongation.[3]

In addition to binding to ribosomal RNA, aminoglycosides have also been found to interact with a variety of other biologically relevant RNA sequences, such as the hammerhead ribozyme,[4] group I introns,[5] the HIV-1 RRE transcriptional activator region.[6] Since the potential of RNA as a new drug target has recently come to the fore,[7,8] methods to measure RNA–small molecule interactions are needed. Fluorescence depolarization[9] and fluorescence anisotropy[10] methods have been proposed to determine the specificity of aminoglycosides–RNA binding. The purpose of this work was to study the aminoglycoside–nucleic acid interactions by a rapid and simple method enabling quantitative binding measurements. We have recently proposed an HPLC method for the measurement of polyamines and lipidic amines binding to DNA,[11] which has also found application for the study of other low molecular weight compounds–DNA interactions.[12–14] This HPLC method, based on DNA or RNA peak size exclusion, was used to study the binding of aminoglycosides to these nucleic acids.

Materials and Methods

Deoxystreptamine dihydrobromide, neamine and the synthetic derivative BKN3 as hydrochloride salts were prepared as previously described.[15] Kanamycin A sulphate was kindly offered by HELP Pharmaceutical Co. (Athens, Greece) and netilmicin sulphate by Shering-Plough Corp. (Kenilworth, NJ). Neomycin B, gentamicin A and streptomycin as sulphate salts, calf thymus DNA (activated type XV) and wheat germ tRNA (type V) were purchased from Sigma Chemical Co. HPLC-grade solvents were obtained from Labscan Ltd (Dublin, Ireland).

A Hewlett-Packard HPLC series 1050 and a Lichrospher RP-18 (250×4 mm, 5 μm) were used. DNA and RNA solutions (0.1 g/L) were prepared in water and kept at 4 °C. Aminoglycosides as hydrochloride or sulphate salts were dissolved in water (each at 0.5 g/L). The column was equilibrated with an H_2O/MeOH (80:20, v/v) solution. Test samples and DNA or RNA solutions at an equivolume ratio were then introduced into the sample loop (20 μL) without incubation. The flow rate was maintained at 1 mL/min, and the free DNA or RNA eluted from the column in approximately 1 min. After the appearance of DNA or RNA peak, the col-

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umn was later washed with methanol for 20 min to elute the sample mixture. DNA or RNA binding is expressed as a percentage of nucleic acid peak exclusion. All samples were tested in triplicate at a final concentration of 35 μM versus DNA or RNA at 0.05 g/L.

Results and Discussion

The structures of aminoglycosides tested are shown in Figure 1. The results of the effect caused by the aminoglycosides on tRNA and DNA are presented in Table 1. All the compounds were tested at a final concentration of 35 μM.

The study of the aminoglycoside antibiotics binding to tRNA is important,[16] because tRNA comprises of the majority of the soluble cytoplasmic RNA. 2-Deoxystreptamine (**1**, DOS), the structural component of the aminoglycosides, exhibited lower tRNA peak exclusion in comparison with the pseudodisaccharide neamine (**2**), a part of neomycin B. Neamine, in turn, showed lower affinity to tRNA than neomycin B (**4**). BKN3 (**3**), a synthetic analogue of neamine, which contains the amino acid phenylalanine at the C-6′ position of the sugar moiety, presented the highest ability for tRNA binding among all the compounds tested. Aminoglycosides are divided into distinct chemical classes: the 4,5-disubstituted and 4,6-disubstituted DOS derivatives represent the two largest subclasses. Neomycin B (**4**), a representative example of the former subclass, binds very strongly to tRNA in comparison with kanamycin A (**5**), gentamicin A (**6**) and netilmicin (**7**), which belong to the later subclass. Among the 4,6-disubstituted DOS derivatives, gentamicin A (**6**) showed the lowest affinity to tRNA. Streptomycin (**8**), a representative example of another subclass characterized by guanidines, with carbamidoyl groups attached to the amino groups in the aminocyclitol ring, caused slightly lower tRNA peak exclusion than kanamycin A and netilmicin.

Neomycin B and the synthetic derivative **3** exhibited a complete interaction with DNA (100%) at a concentration of 35 μM. All the other compounds tested

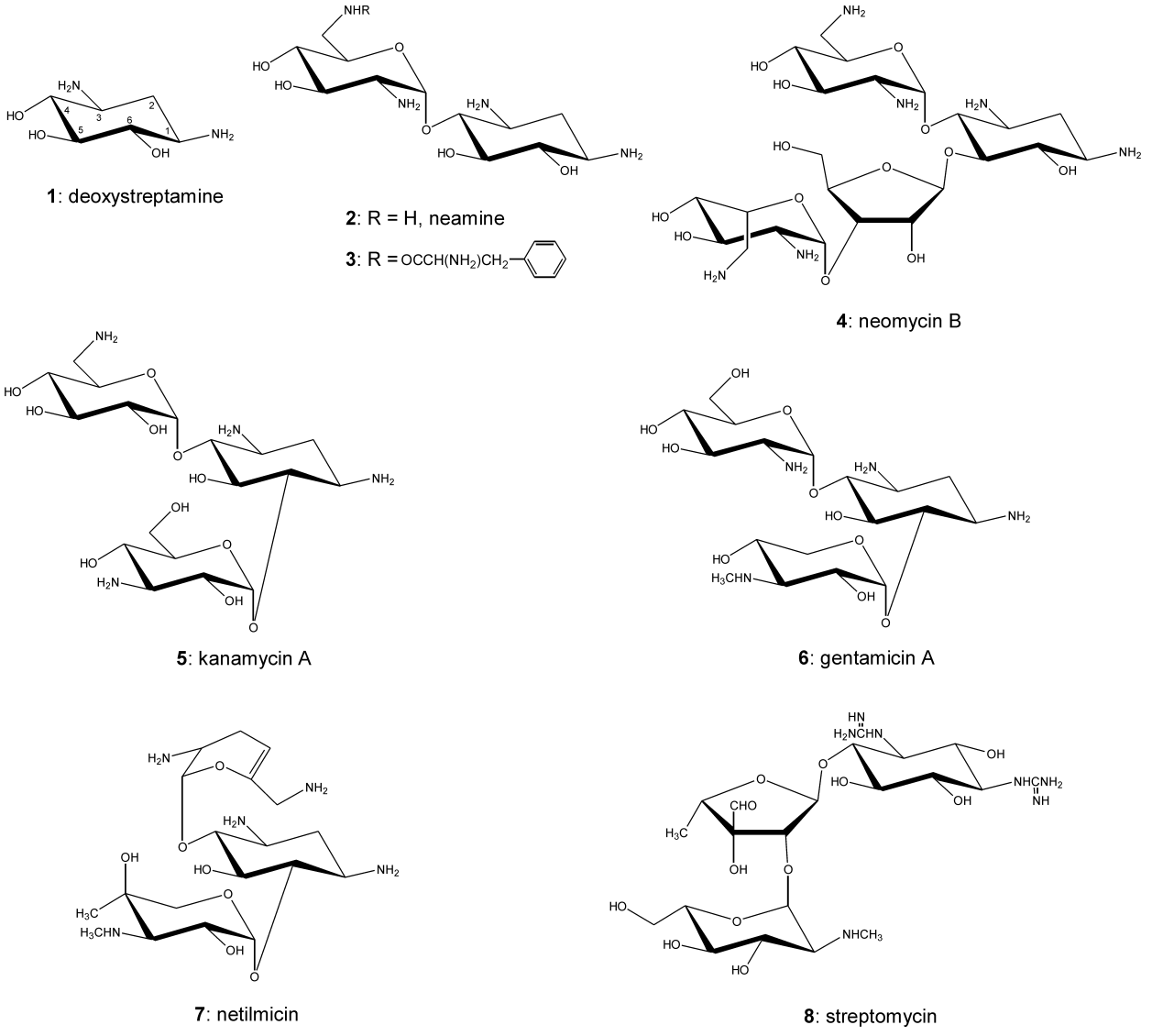

Figure 1. Structures of aminoglycosides studied.

Table 1. Effect of aminoglycosides on tRNA and DNA

No.	Compounds	% tRNA peak exclusion[a]	% DNA peak exclusion[a]
1	Deoxystreptamine	30.3±3.7	—
2	Neamine	47.3±3.5	40±9.1
3	BKN3	100	100
4	Neomycin B	77.8±2.1	100
5	Kanamycin A	56.0±7.9	67.3±10.8
6	Gentamicin A	25.0±3.5	20.3±3.8
7	Netilmicin	55.1±6.3	68.8±7.1
8	Streptomycin	52.5±8.5	44.3±8.5

[a]Mean values of three experiments.

in this study proved to be weaker binders than them. Neamine caused a 40% peak exclusion. Comparing the results for compounds **2** and **3**, it should be noticed that the presence of phenylalanine facilitates the DNA binding. This finding is in full accordance with our previous result.[13] Kanamycin A (**5**) and netilmicin (**7**) exhibited equal activity, while gentamicin A was the weakest binder, as in the case of tRNA. Streptomycin (**8**) presented considerably lower ability for DNA binding than kanamycin A and netilmicin.

The binding results indicate that the affinity of the tested aminoglycosides to tRNA is different than that to DNA. Aminoglycoside antibiotics, due to their polycationic character, are expected to possess a high inherent affinity for the polyanionic nucleic acids. It is likely that aminoglycosides bind to nucleic acids through electrostatic interactions between their positively charged amino groups and the anionic phosphate groups of the nucleic acids backbone. Although the ionic interactions seem to play the predominant role in the nucleic acid–aminoglycoside interactions, it is also possible that hydrogen bonds could have a significant contribution. In addition, other groups, such as the aromatic ring of phenylalanine, may contribute to the overall binding free energy.

We have shown that natural polyamines, as spermine, spermidine, etc., cause a strong DNA peak exclusion effect and that this phenomenon increases when the number of amino groups increases.[11] However, the present study on cationic aminoglycoside–nucleic acid binding can not come to the same conclusion. This result may be understood from the structural properties of the oligocations. Natural polyamines are flexible molecules, while aminoglycosides carry the positive charges to sugar rings that have only a limited number of conformations. Although free rotation around the single bonds that connect the sugars allow aminoglycosides to adapt themselves to many different nucleic acid structures, in most cases it is not possible to have sufficiently close contact between the ammonium ions and functional groups of the nucleic acid.

Up to now the studies carried out focused on the aminoglycoside–RNA interactions.[17] To our knowledge this is the first time that aminoglycosides binding to double helix DNA has been studied. Most recently the stabilization of DNA triple helix by aminoglycoside antibiotics has been reported.[18]

In conclusion, the present method is a rapid and accurate test for the study of aminoglycoside–nucleic acid interactions, which may be used for two applications. First, to determine how specific the binding of a particular aminoglycoside to various RNA sequences is. Second, to design aminoglycoside analogues, specific for a particular RNA structure. Thus, the nucleic acid affinity of a number of synthetic aminoglycoside antibiotics produced by combinatorial or non-combinatorial[19] techniques may be easily studied.

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