shape (see Figure). Other important features of this loop are the base triple U16-C18-G110 (shown in orange) and the U-turn U16 to C18. The upper asymmetric internal loop forms a series of S-turns that span residues C5 to G13. Both of the internal loops zipper up and stack with the central stem, forming a tight structure surrounding the streptomycin binding pocket, which is located in the elbow of the L shape. The tight interlocking of both the upper and lower internal loops is stabilized by a magnesium ion interacting with residues U10-11 from the upper loop and residue C109 of the lower loop (indicated as M1 in the Figure). Residue C109 itself is involved in a noncanonical base pair with G12.

The antibiotic binding pocket is an elaborate structure in which walls are formed by bases from both interlocked loops. The streptose ring of streptomycin is buried deeply in the pocket and makes contacts with multiple residues, in particular residues at positions U11 and G12 from the upper loop and residues U16 and U17 from the lower loop. In contrast to the streptomycin-ribosome structure, most RNA-antibiotic contacts in the aptamer involve base edges and not backbone phosphates. Recognition between the antibiotic and the RNA is predominantly achieved through hydrogen bonds, one of which is mediated through a bridging water molecule. All of the NH_2, NH, and OH groups on the streptose ring are involved in intermolecular contacts, in contrast to the two other streptomycin rings, which are positioned outside the pocket and contribute to binding only through one hydrogen bond. The guanidinium group of the streptose ring is buried most deeply in the binding pocket and is involved in several hydrogen bonds. The substitution of this group by a carbamino group in bluensomycin is the reason for the tight aptamer discrimination between both antibiotics.

This structure demonstrates once more the diversity of RNA ligand interactions. While aromatic ligands like ATP, FMN, and theophylline stack between bases, streptomycin lies perpendicular to the base pair planes. Contrary to previously published aminoglycodise-aptamer structures, neomycin and tobramycin, where the antibiotics lie in the deep groove of a perturbed double helix, streptomycin is locked in place via the two intertwined asymmetric internal loops [4].

Many of the in vitro-selected aptamers adopt their final fold only after ligand binding, with the ligand being an essential part of the structure. In the absence of the ligand, the RNA is rather unstructured. This ligand-dependent structural stabilization prompted the design of a translation regulation system. Aptamers were inserted into the 5′ untranslated leader of messenger RNAs without affecting their expression. Only after addition of the ligand did the RNA fold, leading to repression of translation [7]. Since this discovery, many of us have wondered why nature did not make use of such a clever mechanism. Several years since researchers developed this regulatory concept, it was discovered that this mechanism is indeed used by nature. Metabolite binding domains in mRNAs, which refold after ligand binding, were recently found for cyanocobalamin, thiamine, and FMN [8]. These recent findings give us a taste of what is waiting to be discovered and clearly show that metabolite-RNA complexes will be used in the future for a yet unpredictable number of applications. We can now predict that many biosynthetic pathways will be regulated by metabolite binding "natural aptamers," and we might even find a structure similar to the streptomycin aptamer in a bacterium producing streptomycin.

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Resisting Bacterial Drug Resistance

In this issue of *Chemistry & Biology*, Wright and colleagues report an elegant method for inhibiting enzymes critical for rendering bacteria drug resistant. By using cationic peptides as inhibitors, the authors have exploited two antibacterial mechanisms, making it doubly difficult for microbial retaliation.

Aminoglycosides are one of the oldest classes of antibacterial natural products [1]. These compounds kill bacteria by binding tightly to the acceptor site (A site) on the 30S subunit of the ribosome and consequently inhibit bacterial protein synthesis. As is the case for all the other compounds classes of antibacterials, resistance to these drugs has increased rapidly with usage. In aminoglycoside-producing organisms, resistance to the compound results from the methylation of nucleotides at the A site in the ribosome, preventing the drug from binding due to steric and electrostatic interference. For the notorious pathogen *Mycobacterium tuberculo-*

sis, resistance results from single nucleotide changes in the 16S rRNA of the ribosome (this organism has only a single copy of the corresponding gene) or the S12 small ribosomal subunit protein [2]. In addition, drug efflux is a newly discovered mechanism that is certain to become more relevant to aminoglycoside resistance [3]. However, for essentially all other microorganisms, high-level clinical resistance results from the expression of genes with one of three catalytic activities: ATP-dependent O-phosphorylation, O-nucleotidylation, or acetyl-CoA-dependent N-acetylation [4]. These enzymes covalently modify the drug, resulting in compounds with substantially weaker affinities for their ribosomal RNA target and, ultimately, in drug resistance [5]. In the article by Wright and colleagues published in this issue, the authors have taken a tantalizing step closer to "resisting resistance" [10]. The authors have focused their research on members of both the aminoglycoside O-phosphotransferase and aminoglycoside N-acetyltransferase families for almost a decade and have applied a combination of approaches to dissect these enzymes' structure, function, and mechanism. Particularly noteworthy in this regard was this group's observation that these antibiotic-modifying enzymes are functional and structural relatives of mammalian serine/threonine kinases and histone acetyltransferases [6, 7], and in the case of the aminoglycoside O-phosphotransferase, wortmannin, a known inhibitor of protein kinases, was shown to inhibit the activity of the enzyme [8]. Since both the mammalian homologs of aminoglycoside O-phosphotransferases and N-acetyltransferases naturally act on proteins, the key question was whether peptides could be identified that would bind to the aminoglycoside modifying enzymes and inhibit their activity.

The chemical structures of the aminoglycosides gave a strong hint that positively charged peptides would be the best inhibitors. Furthermore, the three-dimensional structures of both aminoglycoside N-acetyltransferases and O-phosphotransferases revealed a highly negatively charged active site which would most likely accommodate these polycationic antibiotics (remember they bind with nanomolar affinity to rRNA). In addition, a number of cationic peptides have been identified from vertebrates and insects that have antibacterial activity, including the cecropins, pyrrhocoricins, maganins, and indolicidins (reviewed in [9]). Many of these peptides exert their antibacterial effects by binding to the lipopolysaccharide outer membrane component of gram-negative organisms and either forming pores or gaining access to the cytoplasm, where they act on other targets. In this study, a number of these cationic antimicrobial peptides were tested as inhibitors against two different aminoglycoside N-acetyltransferases (AAC(6′)-Ii and e) and two different O-phosphotransferases (APH(2′)-Ia and APH(3′)-IIIa). These results demonstrated a significant degree of selectivity, with certain peptides exhibiting striking inhibitory specificity against a single enzyme, and others showing a broader range of inhibitory activity. They also differed in the type of inhibition that was observed, with some peptides exhibiting competitive inhibition versus aminoglycoside substrate (e.g., AAC(6′)-Ii), and others exhibiting noncompetitive inhibition versus aminoglycoside substrates, suggesting that alternative binding modes are present that are both peptide- and enzyme-dependent. This will only be resolved by elucidation of the high-resolution structures of the complexes.

Using the indolicidin analog CP10A as a starting point, the authors prepared a series of truncated peptides and measured their inhibitory activity against both the N-acetyltransferases and O-phosphotransferases. They were able to clearly define the N-terminal portion of the peptide as the most critical portion required for binding; however, surprisingly, they also showed that the position of the charged residues within the peptide was relatively unimportant in determining inhibitory strength. These synthetic derivatives of CP10A open the way to a more detailed analysis of structure-activity relationships within a defined peptide scaffold. Whether these synthetic peptides also exhibit antibacterial activity remains to be determined.

This work presents an obvious paradigm for developing clinical inhibitors of aminoglycoside-modifying enzymes. The coadministration of a β-lactam and β-lactamase inhibitor (e.g., Augmentin) is extremely effective in treating bacterial infections that do not respond to a β-lactam antibiotic alone. Nearly 100 different aminoglycoside-modifying enzymes have been described that are both laboratory and clinically derived. In addition to the three classes of modifying enzymes, members of each family exhibit an absolute regioselectivity in their modifying activity. This initially implies that overcoming resistance by inhibiting the modifying enzymes would be a nearly impossible task. However, the highly negatively charged aminoglycoside binding sites of both the N-acetyltransferases and O-phosphotransferases and the similarities observed between these two classes of aminoglycoside-modifying enzymes with protein kinases and histone acetyltransferases suggested to the authors that cationic peptides might exhibit both tight binding and, more importantly, a broad spectrum of inhibitory activity against many, or all, aminoglycoside-modifying enzymes. They have shown this conclusively and elegantly.

Finally, as noted earlier, bacteria will inevitably find ways to generate resistance to toxic, bactericidal compounds. However, the combined activity of cationic peptides as inhibitors of aminoglycoside-modifying enzymes and their intrinsic antibacterial activity argues that resistance to these compounds is unlikely to occur in a single genetic transformation. Any genetic event that would reduce the peptide's affinity for its aminoglycoside-modifying enzyme target may reduce the efficacy of the aminoglycoside as an enzyme substrate but, importantly, would leave the intrinsic antibacterial activity of the peptide unchanged. Similar arguments can be made for mutations affecting the target of the antibacterial action of the cationic peptides. Therefore, multiple independent mutations would be necessary to generate insensitivity of the peptide for both its intrinsic target and aminoglycoside-modifying enzyme. Statistically, such an event is extremely unlikely.

The discovery and development of therapeutic antibacterial compounds by the academic and pharmaceutical communities ranks as one of the most important contributions to human health in the last 100 years. The enormous increase in clinical resistance to all classes of antibacterial compounds in the last decade has

shown us that we should not rest on the laurels of past successes, but continue to pursue the development of new classes of compounds effective against bacterial infections. Certainly, the work of Wright and colleagues constitutes a significant step in that direction.

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