SELECTIVE FUNCTIONALIZATION

Shields for small molecules

Nucleic acid aptamers have been employed to shield small molecules so that one among many similar reactive functional groups can be modified. This provides access to new chemical entities with potentially interesting properties while avoiding the use of covalent protecting groups.

Scott K. Silverman

Protecting groups have a long history in synthetic chemistry[1]. A protecting group is covalently attached to one functional group to block its reactivity while a second functional group undergoes a chemical transformation, after which the protecting group is then removed (Fig. 1a). The use of protecting groups requires both protection and deprotection steps, so efficiency considerations dictate that their use should be avoided whenever possible. Indeed, many research groups have sought to develop highly selective synthetic reactions that obviate the need for them[2]. Nevertheless, many situations warrant judicious use of protecting groups, often because the selective chemical methods to circumvent their employment have yet to be discovered. In this context, a report by Andreas Herrmann and co-workers in *Nature Chemistry*[3] describes an intriguing new strategy in which nucleic acid aptamers are employed as noncovalent protecting groups in the form of 'aptameric protective groups', or APGs, for use with otherwise-unprotected complex small-molecule compounds.

What is the motivation for developing APGs? Structurally complex natural products often display numerous chemically equivalent (or nearly so) functional groups that are challenging to differentiate using small-molecule reagents. For example, the aminoglycoside antibiotic neomycin B has six primary NH_2 groups (Fig. 1b), all of which can react with acylating reagents. Preparing site-specifically acylated analogues of neomycin B by direct reaction with acylating reagents is generally intractable. Herrmann and co-workers reasoned that complexing neomycin B with a macromolecule that shields most of the NH_2 groups would allow a remaining, unshielded NH_2 group to react preferentially, thereby enabling efficient synthesis of various site-specifically modified analogues of neomycin B without resorting to total synthesis or other approaches. A schematic depiction of the APG strategy is shown in Fig. 1c.

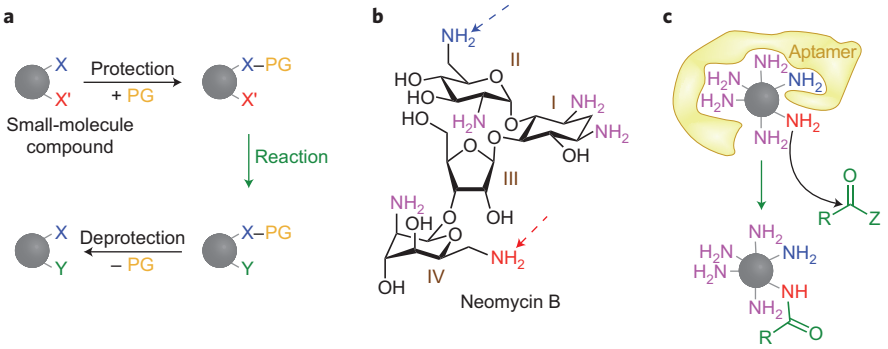

Figure 1 | Protecting groups and the new aptamer-based strategy for selective chemical modification. **a**, Conventional use of a protecting group (PG). **b**, Neomycin B, the aminoglycoside antibiotic used by Herrmann and co-workers to demonstrate their new strategy[3]. Rings I–IV are numbered. The two most reactive NH_2 groups with acylating reagents (out of six total) are marked with arrows. **c**, The 'aptameric protective group' strategy. The neomycin B aptamer physically shields the four NH_2 groups on rings I and II and partially shields one of the two NH_2 groups on ring IV. In the presence of the aptamer, only one of the two NH_2 groups on ring IV is reactive; which of the two reacts depends on the small-molecule reagent.

For this approach to work, an appropriate macromolecule must be identified to serve as a noncovalent protecting group. Supramolecular complexes have been used for this purpose[4–6], but without any clear path to generalize their application. Fortunately, there is another way. In the early 1980s, the discovery of naturally occurring catalytic ribonucleic acid (RNA), or ribozymes, established experimentally that nucleic acids can do more than store genetic information[7]. Soon thereafter, artificial RNA aptamers that bind various small-molecule compounds (and later proteins) were identified by *in vitro* selection[8]. The more recent discovery of gene-regulating 'riboswitches'[9] showed that nature also uses RNA for important binding functions. Both binding and catalysis require the formation of a three-dimensional tertiary structure — a situation similar in concept (if not in detail) for RNA and proteins. As RNA is proficient at binding small-molecule compounds, Herrman and co-workers proposed that aptamers can serve as noncovalent protecting groups.

Although some aptamers completely engulf their small-molecule targets, especially compounds of very low molecular weight, others bind to only a portion of the molecule[10]. Therefore, in their new APG strategy, Herrmann and co-workers exploited previously identified RNA aptamers known to bind neomycin B. In an acylation reaction with the *N*-hydroxysuccinimidyl ester of acetic acid, non-aptamer-protected neomycin B led to a mixture of products with acylation at all six NH_2 groups. Under conditions optimized for monoacylation, the reaction led to an inseparable ~1:1 mixture of acylation products at the two most reactive NH_2 groups, which are both attached to primary carbon atoms (marked blue and red in Fig. 1b). However, when an RNA aptamer that is known to bind to rings I–III of neomycin B was present, only a single NH_2 group from ring IV reacted (marked red in Fig. 1b), with >70% conversion and ~20:1 site-selectivity between the two most reactive NH_2 groups, even in the presence of 30 equivalents of the reactive ester reagent. Further experiments with an aromatic isocyanate instead of an activated ester as electrophile also led to highly site-selective modification of ring IV, but with reaction now occurring at the nearby NH_2

group attached to a secondary carbon atom. The distinct chemical properties of the two electrophiles were invoked to explain these different sites of reactivity. Several of the modified neomycin B derivatives retained antimicrobial activity against *Escherichia coli*, demonstrating the value of the APG approach for evaluating functional characteristics of the new chemically modified products.

This new aptamer-based APG strategy has several advantages for future applications. The APG technology may allow even highly complex natural products to be used as lead compounds for direct derivatization as part of drug discovery efforts. The time and effort required to identify and characterize a suitable aptamer may be justified because the synthetic expenditure is obviated, especially for compounds such as neomycin B that would otherwise require a lengthy synthetic route. In this context, it is notable that Herrmann and co-workers prepared six different neomycin B derivatives using a common APG approach, suggesting that, in the general case, numerous derivatives of a single natural product should be accessible in parallel fashion. Moreover, an aptamer developed for one natural product will probably be useful as an APG for derivatization of structurally related compounds. Herrmann and co-workers have demonstrated this explicitly by synthesizing a derivative of paromomycin (a neamine antibiotic like neomycin B) using a neomycin B aptamer. All seven of the aminoglycoside antibiotic derivatives prepared using the APG approach — in merely one step each — are entirely new compounds that would require lengthy chemical synthesis (>20 steps) if prepared conventionally[11].

Some practical concerns must be faced before this new aptamer-based APG strategy can be applied more broadly. The derivatization reactions are reported only on the micromole scale, which is sufficient for high-performance liquid chromatography separation and NMR characterization of the products. The limiting reagent for scale-up is the aptamer itself, and although oligonucleotides can be synthesized in much larger quantities, cost becomes an issue. Using deoxyribonucleic acid (DNA) rather than RNA aptamers would be helpful, considering that DNA is about an order of magnitude less expensive than RNA, and also noting that DNA has no apparent binding disadvantage relative to RNA[12]. In any case, complex molecules of interest for the APG approach — such as aminoglycoside antibiotics, among others — can probably be studied even on the micromole scale; for example, in bioassays as performed by Herrmann and co-workers.

A more thorny concern is that a suitable aptamer must either already exist or be newly identified and characterized for any desired small-molecule derivatization target. Herrmann and co-workers used three previously reported RNA aptamers for neomycin B, but for many other molecules it is likely that no pre-existing aptamer is available. Although *in vitro* selection of RNA and DNA aptamers ('SELEX') is now routine[8], the aptamer selection and characterization process is not readily performed using equipment and expertise available in most synthetic laboratories. Aptamer identification itself is not always successful, despite implications in many publications and reviews. Finally, not just any aptamer will suffice; to be an effective APG, the aptamer must shield most functional groups for which reactivity is undesired, leaving primarily the intended functional group accessible for reaction. I anticipate that identifying and characterizing suitable aptamers will be the rate-limiting step for widespread adoption of the APG approach.

In summary, the report by Herrmann and co-workers[3] clearly validates the concept that aptamers can be highly effective noncovalent protecting groups. Their unconventional combination of nucleic acid technology and synthetic chemistry is an intriguing and promising strategy for site-selective covalent modification of structurally complex small-molecule compounds. ❐

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CHEMICAL VIROLOGY

Packing polymers in protein cages

The combination of addressable synthetic macromolecules with proteins of precise structure and function often leads to materials with unique properties, as is now shown by the efficient multi-site initiation of polymer growth inside the cavity of a virus capsid.

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Biological nanostructures, such as enzyme clusters and viruses, are typically superior to synthetic nanoscale materials when it comes to their degree of sophistication in terms of both structure and function. It has, therefore, been a rewarding strategy to combine these biomacromolecules with synthetic polymers to obtain hybrids that combine the properties of both types of material[1]. In recent years, the use of virus capsids — the protein cage of a virus from which the genetic cargo has been removed — has emerged as a way to construct (semi)biological nanostructures.

One of the reasons that protein cages, either derived from viruses or other sources (such as ferritin, heat-shock proteins and vaults) have attracted so much interest is their high degree of ordering: an exact number of proteins assemble with cubic symmetry. Because of this symmetry, functional units (for example, amino acid residues or pores) are present on precisely located positions within the cage