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Point of Attachment and Sequence of Immobilized Peptide-Acridine Conjugates Control Affinity for Nucleic Acids

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Combinatorial chemistry has become a powerful tool for the discovery of small molecules with potential applications both as therapeutic agents and as new research tools.[1] A reliable high-throughput assay is essential to screen libraries for functional molecules. One successful strategy involves spatially arraying library members either on solid-phase synthesis beads[2] or on glass plate microarrays.[3] A potential limitation of spatial array screening, however, is that attachment of the small molecule to a solid support might inhibit its binding.

We have synthesized libraries of peptide-acridine conjugates (PACs) featuring a novel 9-anilinoacridine amino acid that we wish to screen for high-affinity RNA ligands using spatial arraying strategies.[4] However, it is not currently known if immobilization of a PAC affects binding to RNA targets. Similar compounds have been shown to bind nucleic acids by threading intercalation.[5] Because one end of these molecules must pass between base pairs to allow maximum stacking of the acridine intercalator, linkage of this threading substituent to a solid support may significantly slow binding or prevent it altogether. Various classical intercalators have been immobilized for use in affinity chromatography applications.[6] Yet, an analysis of how the point of attachment or substituent structure of a solid-supported intercalator affects nucleic acid binding has not been reported. In this communication, we describe an experimental approach to probe these effects on the binding of solid-phase immobilized PACs to TAR RNA. The results have implications for both on-bead and microarray-based selections and in understanding the nucleic acid binding of functionalized intercalators.

To compare two PACs of the same sequence, which differ only in their site of attachment to a solid support, we devised a scheme to immobilize the compound via reductive amination. Initially, we used the peptide sequence Asn-Val-*Acr*-Ser-Tyr, where *Acr* is our 9-anilinoacridine amino acid.[4] For C-terminal attachment, a lysine residue was incorporated prior to coupling of tyrosine, and the N-terminus was capped with an acetyl group. Following cleavage and HPLC purification, the ϵ-amino group of lysine was coupled to a commercially available aldehyde resin to afford the PAC-C support (**1**, Figure 1).[7] For comparison, γ-aminobutyric acid was installed at the N-terminus after asparagine, and the primary amino group of this amino acid reacted under the same conditions to yield the PAC-N support (**2**, Figure 1).

To assay for nucleic acid binding, each resin was incubated with radiolabeled TAR RNA. This 59-nt RNA sequence folds into a hairpin stem with extensive duplex structure.[8] Because it is known that acridines are fully displaced from duplex nucleic acids at high salt concentrations, binding stringency was increased by increasing the ionic strength in the elution buffer.[9,10] As a result, TAR was

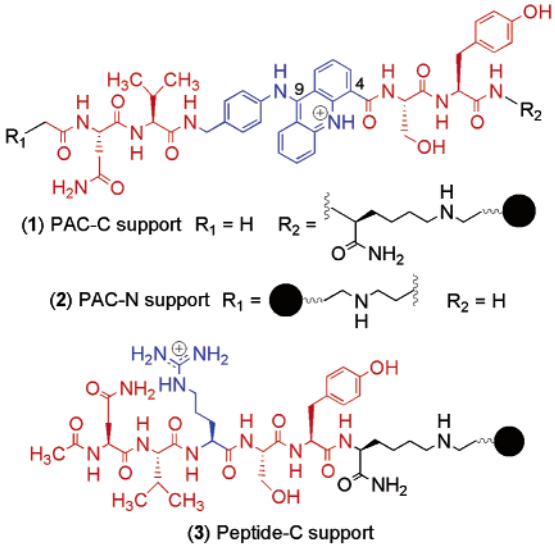

Figure 1. Structures of three solid supports used in this study.

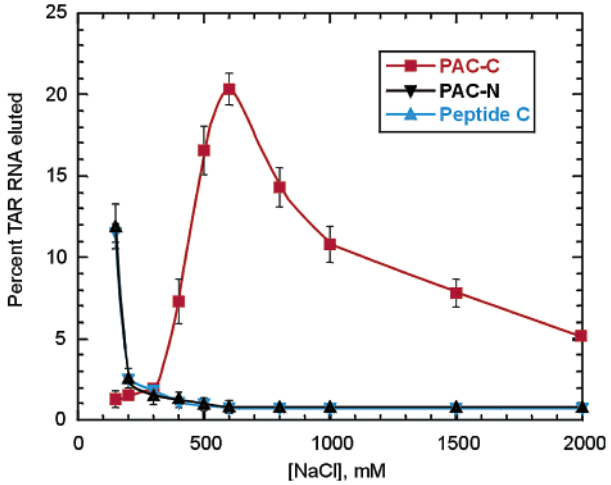

Figure 2. Plot of the percent TAR RNA eluted from PAC-C, PAC-N, and peptide-C supports as a function of salt concentration.

observed to elute much earlier from the PAC-N support as compared to the PAC-C support (Figure 2). Indeed, the majority of the TAR RNA was found in the flow-through fraction with the PAC-N support, whereas 400 mM NaCl was necessary to begin the elution of TAR from PAC-C. Similar results were observed with 30-bp DNA and RNA duplexes, suggesting the mode of binding for PAC-C is not specific to single-stranded regions of TAR and is consistent with intercalation (data not shown).[11]

To directly assess the importance of the intercalator in the peptide sequence, a control resin (peptide-C support, **3**) was constructed

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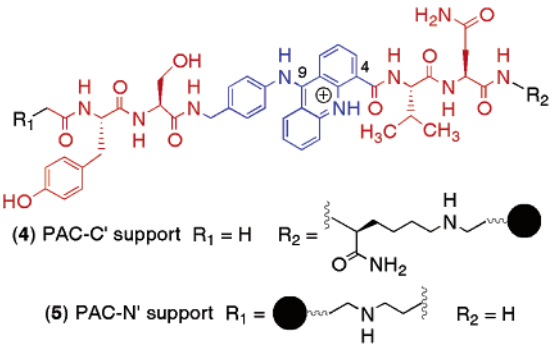

Figure 3. Structures of additional PAC supports used to test the relative importance of polarity and sequence in TAR RNA binding.

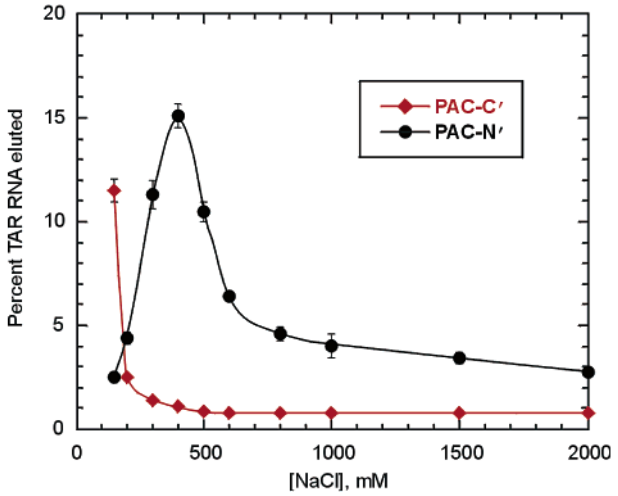

Figure 4. Plot of the percent TAR RNA eluted from PAC-N′ and PAC-C′ supports as a function of salt concentration.

where a positively charged amino acid (arginine) was substituted in place of the acridine. The experiment was repeated with peptide-C, and we observed poor binding of TAR in a manner similar to that of PAC-N (Figure 2). This result indicated that the high-affinity binding to the PAC-C support was dependent on the presence of our 9-anilinoacridine amino acid.

It appeared that solid-phase immobilization of the PAC via its N-terminus inhibited binding. To test whether N-terminal tethering generally blocked PAC binding, two additional resins of a different sequence (Tyr-Ser-*Acr*-Val-Asn) were constructed, yielding PAC-C′ (**4**) and PAC-N′ (**5**) solid supports (Figure 3). Interestingly, we found that the PAC-N′ support, which is attached via its N-terminus, displayed higher RNA-binding affinity than the C-terminally tethered PAC-C′ (Figure 4). Thus, with the sequence reversed, the preferred point of attachment changed. Therefore, we conclude that the point of attachment of an immobilized PAC and its sequence together control its affinity for nucleic acids.

The origin of the effect PAC sequence has on nucleic acid binding is unknown at this time. Given the available high-resolution structures of complexes formed between substituted acridines and duplex nucleic acids, side chains stemming from the 4- and 9-positions are believed to be simultaneously localized in the two different grooves of a duplex with the acridine stacked between base pairs.[12] Thus, the effects observed here may be kinetic and/or thermodynamic in origin. The kinetics may be altered if a sterically demanding peptide structure is required to pass between base pairs to form the final intercalation complex. However, the formation of stabilizing or destabilizing interactions between functional groups in the PAC side chains and the grooves of the nucleic acid target may also modulate the thermodynamic stability of the complex. Additional experiments are required to reveal the relative importance of these parameters on the nucleic acid binding of immobilized PACs.

In summary, we have developed a simple method to immobilize PACs of varying sequence and assay for nucleic acid binding. We have shown that both the amino acid sequence of the PAC, as well as its point of attachment to the solid support, are important in determining affinity for nucleic acids. It is evident from these experiments that when immobilizing PACs for on-bead or microarray-based selections, both C- and N-terminally tethered libraries should be generated, because the binding of certain sequences may be inhibited by one linkage but not the other. Furthermore, these results highlight the potential importance of utilizing multiple tethering strategies when screening other spatially arrayed libraries for ligands to complex biomolecules.

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Supporting Information Available: Experimental procedures for solid-phase synthesis of PACs, HPLC/MS characterization, attachment to the solid support, preparation of nucleic acids, and the solid support binding assay (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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