In Vitro Selected Oligonucleotides as Tools in Organic Chemistry

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new tools

Abstract: DNA and RNA molecules have been isolated from synthetic combinatorial libraries that are able to bind a variety of small organic molecules and catalyze different chemical reactions. The properties of these molecules as well as the techniques for their identification are discussed with respect to potential applications in organic synthesis.

Key words: RNA catalysis, in vitro selection, aptamers, ribozymes, combinatorial chemistry

1 Introduction

For a long time, nucleic acids have been the subject of intensive biochemical studies, and oligonucleotides as short synthetic pieces of DNA have become essential research and diagnostic tools in molecular biology. For the organic chemist, however, oligonucleotides have been more a subject of synthetic investigations than tools that help to solve synthetic problems. Elaborate solid-phase methodologies were developed, and now automated synthesizers allow the reliable synthesis of DNA and short RNA oligonucleotides as well as the site-specific incorporation of numerous modifications.

With the advent of combinatorial chemistry in the early 1990s, the laboratories of Gold, Szostak and Joyce were the first to demonstrate that one can isolate from synthetic combinatorial RNA or DNA libraries molecules with new properties, which are not related to the biological functions of nucleic acids.[1-3] The most prominent examples are nucleic acids with new binding properties and those with novel catalytic activities. Nucleic acid ligands were generated against small organic molecules that exhibit remarkable enantioselectivity or highly selective discrimination between closely related molecules. The properties of the isolated molecules (e.g., the specificity of binding) could be largely determined by the design of the selection experiments. In similar studies, a great many of new catalysts were generated. While the first examples dealt with modifying reactions of nucleic acids, recent work has created catalysts for Diels-Alder reactions and amide bond formation. The possibility to rationally influence the properties of the molecules to be selected makes the iterative search in and the evolution of nucleic acid libraries potentially superior to the screening of other compound libraries. Potentially, nucleic acid molecules derived by in vitro selection could become useful tools for the organic chemist as tailored highly selective ligands for preparative separations or as specifically developed catalysts in bioorganic transformations.

The whole field has been the subject of numerous review articles.[4-7] Rather than adding another one, we want to highlight in this publication the most important selection methodologies and the properties of selected nucleic acid molecules with respect to their potential application in organic synthesis. It must be noted, however, that while several applications to cellular and biomedical problems have been reported, to-date no practical utilization in organic synthesis has been described.

2 Nucleic Acids Seen with the Eyes of a Chemist

Stripped from all biological context, nucleic acids are linear heterocopolymers. They consist of 4 different monomers, the nucleotides. The properties of nucleic acids are governed by the order in which these monomers appear, which is their sequence. Since the nucleotides have the ability to form specific hydrogen bonds with ”complementary nucleotides” (i.e., those with the opposite H-bond donor/acceptor pattern), nucleic acids can form higher-order structures: They can fold back intramolecularly and thereby form structures with double-stranded stems and single-stranded loops and bulges, or they can recognize intermolecularly a complementary piece of nucleic acid and form more complex aggregates. Several other modes of interaction are known like three- and four-stranded structures and pseudoknots which stabilize and tune the three-dimensional structure necessary to present the functional groups in the proper arrangement to interact with a variety of organic molecules. By doing so, a remarkable variety of different shapes is created. Like in proteins, the three-dimensional architecture of folded nucleic acids may provide binding pockets and catalytic centers. The physico-chemical properties of individual functional groups (e.g., pK_a values) can change tremendously in the context of a specific three-dimensional structure. Various types of interaction with small organic molecules are known.[8,9] Aromatic rings can intercalate between adjacent nucleobases thereby participating in the nucleobase stacking. Heteroatoms of organic molecules can form dipolar contacts with the hydrogen donors and acceptors of the oligonucleotides, especially the heteroatoms of the nucleobases and the 2’-hydroxyl groups of the ribose moieties. The negatively charged phosphate groups of the oligonucleotide backbone can form electrostatic contacts either directly with cationic groups present in the target molecule or via a suitable positioning of divalent metal ions.[10]

The modes of interaction can be further expanded if nucleotide analogs containing unnatural functionalities (e.g. thiols, amino groups, imidazolyl moieties, hydrophobic side chains) are used.[11]

Nucleic acid libraries are like any other library, except for the following differences:

– They are very easy to create. In automated solid-phase synthesis, the machine must just be programmed to use a mixture of monomers rather than a specific nucleotide. A position where an equimolar mixture of the 4 monomers is used is called "randomized", and with N randomized positions inside a given sequence, one (theoretically) creates a mixture of 4^N different oligonucleotides.

– The library sizes are huge. In a typical selection experiment, the initial library contains 10^{14} to 10^{16} different molecular species in a single test tube.

– The most important difference is that from any given nucleic acid molecule, enzymes (template-dependent polymerases) can create thousands or even millions of copies. This advantage, which is obviously not available for other classes of compounds, allows the iterative screening of very large oligonucleotide libraries. By this multiple, consecutive purification, the active species present initially only in very few copies (<20) can be enriched and eventually isolated.

The major disadvantage is the restriction to only four, chemically rather similar monomers, which is caused by the need to use enzymes obeying the Watson-Crick base pairing rules for the copying steps.

3 RNA and DNA Molecules with Specific Binding Properties

3.1 In Vitro Selection

The most common method for the isolation of nucleic acids with an affinity to a certain target molecule uses affinity chromatography (Figure 1) with a solid matrix to which the target molecule of interest is immobilized.[12] The combinatorial nucleic acid library is created by auto-

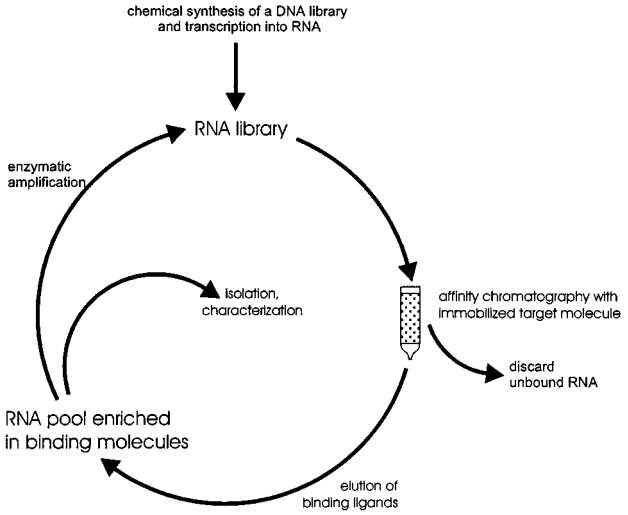

Figure 1. In vitro selection of RNA ligands with specific binding properties.

Biographical Sketches

Andres Jäschke, Christian Frauendorf, Felix Hausch (from left to right).

Andres Jäschke studied Chemistry at Humboldt-University in Berlin. After completing his Ph.D. thesis about oligonucleotide-polymer conjugates under the guidance of Prof. Dieter Cech in 1993, he started working in biochemistry with Prof. Alexander Rich at the Massachusetts Institute of Technology. In 1995 he joined Free University in Berlin, where he is currently heading a research lab. His research interests include RNA catalysis, the application of nucleic acid catalysts in organic synthesis, and new combinatorial strategies.

Christian Frauendorf studied Chemistry at Humboldt-University in Berlin and joined the Jäschke lab in 1996, where he is working on RNA-catalyzed C-N-bond formations.

Felix Hausch studied Chemistry in Darmstadt, Bordeaux, and Berlin where he obtained his diploma in 1996. He is currently completing his doctoral thesis in the group of Andres Jäschke on RNA-catalyzed redox reactions.

mated solid-phase synthesis and then applied to the affinity matrix in a suitable buffer. Unbound nucleic acid molecules are washed away retaining the desired binding molecules which are later competitively eluted with a buffer containing the free target molecule. The eluted molecules are collected, a template-dependent polymerase and nucleotides are added and several thousand copies of each selected nucleic acid molecule are generated. This results in a library in which molecules with affinity to the immobilized target are enriched. Since molecules with high affinity to a given target are very rare, and enrichment of active species in one such round of selection and amplification is normally in the order of 10^2 to 10^3, this scheme has to be repeated several times, until molecules with the desired properties dominate the enriched library. The members of this library are then identified and their nucleotide sequences compared to find common structural motifs.

In vitro selection[1,2,13] (also termed SELEX - Systematic Evolution of Ligands by EXponential enrichment) can be carried out with either DNA or RNA libraries, and modified DNA/RNA libraries can be used too, as long as the chemical modifications do not interfere with the enzymatic amplifications. Technical details of the amplification procedures vary, depending on the type of nucleic acid library used.

The majority of the selection experiments described so far were concerned with oligonucleotide binding to all kinds of bioactive macromolecules, mainly proteins.[14] These oligonucleotide binders, generally called aptamers (*lat.* aptus: to fit), are now entering phases of biomedical and pharmaceutical application and should not be within the scope of this review.[6] The range of small molecules to which binding oligonucleotide sequences have been found comprises biological monomers like amino acids or nucleotides, cofactors like NAD or biotin, natural RNA binders like glycoside antibiotics, transition state analogs, substituted purines or organic dyes. These small target molecules with their corresponding aptamers and binding constants are summarized in table 1.

One of the smallest aptamer motifs was found by Sassanfar and Szostak[19] in a selection experiment against adenosine triphosphate (ATP). They synthesized a DNA pool with 120 randomized positions, transcribed it enzymatically into RNA and applied this RNA pool to a solid matrix derivatized with ATP (Figure 2a). Unbound or only weakly adsorbing RNA molecules were removed by extensive washing. ATP-binding RNAs were then specifically eluted by competition with free ATP present in the elution buffer and enzymatically amplified to yield a new RNA pool enriched in binding sequences which was subjected to further rounds of selection. Starting with about 10^{14} different RNA molecules, only few ATP-binding sequences dominated the pool population after the 8th round. These were isolated and characterized, and a minimal ATP-binding motif was identified based on the obtained sequence information (Figure 2b). This 40mer RNA bound ATP with a K_d of about 14μM. The structure depicted in Figure 2b appears to be a nearly optimal solution for the ATP-binding problem as several independent selection experiments for adenosine containing cofactors subsequently reproduced this motif under varying experimental conditions.[21,33]

Table 1: Small molecule targets of RNA binders (Selection)

Target	K_d [μM][a]	Minimal motif size	Reference
L-valine	12000	-	15
L-citrulline	65	44	16
L-arginine	0.33	-	17
D-arginine	135	38	18
D-ATP	14	40	19
L-adenosine	1.7	58	20
riboflavin	0.5	35	21
NMN/NAD	2.5	-	22
guanine	1.3	32	23
guanosine	32	-	24
7-methyl-GTP	0.5[b]	-	25
theophylline	0.1	38	26
cibacron blue 3G-A (CB)	<100	-	2
dopamine	2.8	-	27
cyanocobalamin	0.088	35	28
biotin	6	-	29
tobramycin	0.009	27	30
neomycin	0.1	-	31
kanamycin, lividomycin	≈0.3	-	32

[a] Binding constants may vary slightly depending on the individual assay conditions (e.g. Mg^{2+}-concentration).
[b] IC_{50} values were determined instead of K_d values.

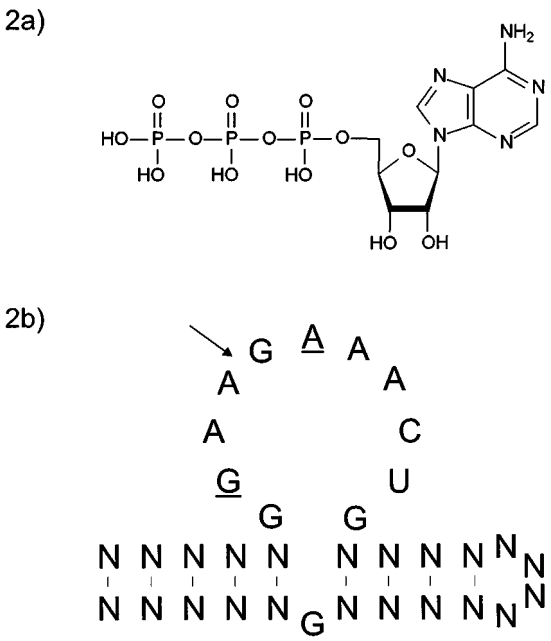

Figure 2. a) ATP and b) the corresponding aptamer. N can be any nucleotide, base-pairing is indicated by thin lines. The underlined nucleotides are involved in hydrogen bonding with the ATP and the arrow marks the intercalation site of the target adenine base.

Subsequent NMR studies of the AT*P*-aptamer complex revealed the detailed molecular interactions between the target and its ligand (Figure 2).[9,34,35] The target adenine base is stacked between two neighboring purines and is hydrogen bonded by two conserved nucleotides in the motif. This structure also explains the remarkable specificity of recognition by the aptamer. Of the four natural nucleotides, only adenosine is recognized while the adenosine derivatives AMP, ADP and ATP are tolerated since these phosphates do not participate in the aptamer-target interactions.

It is, thus, possible to intentionally generate ligands for a desired target. Several major improvements of the selection methodology have been developed since that allows fine-tuning and evolutionary refinement of the selected oligonucleotides.

3.2 Counter Selection – Regioselectivity of Aptamers

In a modified in vitro selection scheme called counter SELEX aptamers can be purposely raised to discriminate between closely related substances.

This was first demonstrated by Jenison et al.,[26] selecting RNAs binding to an affinity column derivatized with the purine analog theophylline (Figure 3). Prior to the competitive elution with free theophylline, the bound RNAs were first incubated with caffeine thereby removing those RNAs capable of binding to both purine analogs. After eight rounds of selection, an isolated RNA bound theophylline with a dissociation constant of 0.32 µM. In contrast, the aptamer bound caffeine only weakly (K_d = 3.5 mM) and therefore could discriminate between the two purines differing by as little as a single methyl group with a factor of 10,900 (Figure 3). By the same technique, aptamers were selected binding the guanosine analog 7-methyl-guanosine triphosphate. The obtained oligonucleotides preferentially bound the guanosine analog a thousand times tighter than the unmodified GTP.[25]

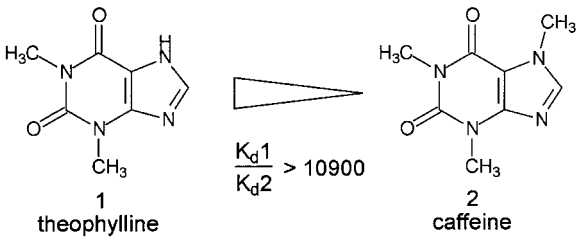

Figure 3. Molecular discrimination of small molecules by an aptamer.

Counterselective pressure is also routinely used in selection experiments to remove RNAs binding to underivatized matrix material by the use of a precolumn. Counter selection can also be used in a multi-dimensional way using more than two different targets to emphasize/de-emphasize several positions in a given structure.

3.3 Stereoselectivity of Aptamers

DNA and RNA - constituted of a (deoxy)ribose phosphate backbone - are chiral polymers occurring naturally only in the poly-D-conformation. This can impose a high sensitivity towards the asymmetrical centers in their target molecules.

In a selection experiment by Klußmann et al.,[20] D-RNA aptamers were selected against the L-adenosine. Counterselection with the D-adenosine was employed to enhance the stereoselectivity of the resulting aptamers. The isolated adenosine binders indeed favored the L-enantiomer about 9000-fold over the D-enantiomer. This was subsequently validated for the corresponding enantiomers of target and ligand, i.e. the chemically synthesized L-RNA aptamer favored D-adenosine 9000-fold over L-adenosine. This rational approach termed 'mirror-image design' extends the range of in vitro selection to the previously inaccessible classes of L-nucleic acids.[18]

It was supposed that high affinity of an aptamer should imply high selectivity since a large number of functional groups are involved in binding interactions.[36] This was demonstrated in an experiment where 20 rounds of selection yielded very tight binders of L-arginine (K_d = 330 nM). The obtained minimal motifs were - as expected - larger and more complex than comparable aptamers with lower arginine affinity and showed excellent discrimination against related compounds. They also exhibited an over 12,000 fold preference over the enantiomer D-arginine.[17]

3.4 In Vitro Evolution

One should keep in mind that most often the initial library contains only a tiny fraction of all theoretically possible pool members. As already stated, with N randomized positions inside a molecule, the theoretical complexity of the library is 4^N. With 25 randomized positions, we get 4^{25} or roughly 10^{15}, which is about the limit of what can be handled with a reasonable amount of work and at reasonable costs. Very often, the length of the randomized part is in the 100 nucleotides range or above, which means that only very few examples (4^{25} out of 4^{100}) are contained in the initial library. This means, on the other hand, that the molecules selected from such an under-represented library are very likely to be not optimal. These ligands, however, can be a good starting point for what is called ”in vitro evolution”.[37,38] While the techniques described so far are simply iterative screening procedures with inserted amplification steps, in vitro evolution adds the element of mutation. The most simple variant uses error-prone amplification. The enzymatic amplification steps are carried out under conditions where the copying enzymes work sloppily, i.e., they create imperfect copies of the templates containing one or several point mutations. This results in new diversity in each round of selection which is now not random but ”biased” in favor of the already enriched molecules.

By using in vitro evolution, the relevant properties (e.g., binding constants) could be improved by several orders of magnitude. In other projects, in vitro evolution was used to change the specificity of a once-identified RNA motif. Famulok isolated arginine binders starting from a library of mutated citrulline binders, and he could show, that the two binding motifs differed only in 3 nucleotides.[16] This technique could therefore be useful to develop binders for molecules, when RNA molecules with affinity for related targets are already known.

4 Ribozymes – New Nucleic Acid Catalysts

4.1 General considerations

While the concept of selecting or generating ligands for a given target can be easily comprehended, the identification of new catalysts is conceptually more ambitious. A catalyst is – by definition – required to leave the reaction unchanged. In order to preparatively separate the few active molecules in a library from the excess of inactive species, however, one needs a difference in the physical or chemical properties. This is a serious contradiction, but two major detours have been worked out.

4.2 Selection Against Transition State Analogs (TSA's)

The concept of transition state stabilization and transition state analogs has been very successfully applied in the field of catalytic antibodies. Antibodies were raised in animals against a transition state analog (TSA), using the animal's immune system as a self-optimizing combinatorial library. Antibodies that bound to these TSA's were then screened to find molecules that catalyze the respective reaction that proceeds via this transition state. This has become a well-established technique, and recently first applications in preparative organic chemistry have been described.[39]

Several groups have tried to apply this concept to combinatorial RNA and DNA libraries. A TSA is immobilized, and nucleic acid ligands are enriched that bind to this TSA. The binding RNAs are then screened for catalysis of the respective reaction. While the first part worked quite often, the isolated RNAs only occasionally displayed catalytic activity. Only two examples are known to-date, an RNA catalyzed isomerization of a bridged biphenyl with multiple turnover,[40] and the metallation of a porphyrin.[41,42] Numerous unsuccessful attempts have been reported.

4.3 Direct Selection

The most successful method to identify nucleic acid catalysts is direct selection. Those members of a combinatorial DNA or RNA library are isolated that show an accelerated reaction with a substrate X (Figure 4), assuming that these members might combine substrate and catalyst properties in one molecule. For selection purposes, X carries an anchor group; a functional group that is normally not present in RNA, like a thiol or biotinyl group. RNA molecules that react with the substrate automatically acquire the anchor group and can subsequently be isolated by affinity chromatography on a suitably derivatized matrix, e. g. activated thiopropyl agarose (disulfide bond formation with the thiol anchor group) or streptavidin agarose (specific interaction with the biotinyl group). Unreacted RNA does not bind to the matrix since it does not contain the anchor group and is removed by washing, while bound RNA is isolated and enzymatically amplified. This cycle is repeated, until active molecules dominate the library.

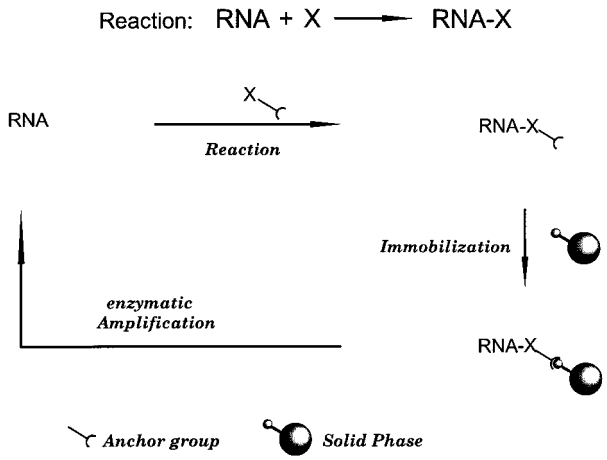

Figure 4. Direct selection of catalytic RNA.

The isolated molecules are self-modifiers and not catalysts in the sense of the definition, they do not leave the reaction unchanged, and they perform the reaction only once. Quite often it is possible, however, to rationally dissect the identified self-modifiers into a substrate part and a catalyst part after elucidation of the primary and secondary structure. E. g., a ribozyme accelerating the transfer of a phosphate group onto its own 5'-end was rationally converted into a true enzyme that is able to phosphorylate external oligonucleotides.[43]

Wilson and Szostak [44] selected self-alkylating ribozymes by using *N*-biotinyl-*N*´-iodoacetyl ethylenediamine as alkylating reagent, in which biotin is the anchor group. They incubated the RNA pool with the alkylating reagent and isolated by affinity chromatography with streptavidin agarose those RNA molecules that had acquired the biotin tag. After several rounds of in vitro selection and evolution they obtained ribozymes catalyzing self-alkylation with a rate enhancement of more than 1 million over the uncatalyzed reaction. The alkylation took place on the N7 imino group of an internal guanosine residue.

In order to increase chances to select active catalysts, these authors did not use a completely randomized pool. They incorporated into their pool design a previously

identified biotin-binding motif, which was partially mutated at a low level and which was surrounded by stretches of completely randomized RNA. This has become a rather common strategy, one first identifies a binding motif for the potential substrate and uses this motif as a starting point in the search for catalysts.

Using these techniques, a great many ribozymes could be selected performing various types of chemical reactions. The range starts from ribozymes accelerating the reactions catalyzed also by the naturally occurring ribozymes (i. e., phosphoester transfer reactions), to alkylation, amide bond formation and acylation reactions. Other impressive examples of the catalytic performance of RNA include the formation of *N*-glycosidic bonds [45] and various aminoacyl transfer reactions [46-49] (for a selection, see Table 2).

Table 2: Selection of organic reactions catalyzed by oligonucleotides

catalyzed reaction	rate acceleration	reference
phosphorylation	100,000	43
RNA ligation	700	57
RNA cleavage	a	58
DNA cleavage	1,000,000	59
aminoacylation	>100,000	46
aminoacyl transfer	>2,000	47
porphyrin metallation	460	42
	1,400	41
biphenyl isomerisation	88	40
N-alkylation	> 1,000,000	44
S-alkylation	2,420	60
N-glycosidic bond formation	10,000,000	45
transesterification	a	48
amide bond formation	1,000,000	49
	10,000	51
Diels-Alder reaction	800	50
	20,000	53

[a] The rate acceleration could not be measured due to undetectable background reaction

Despite the success of direct selection, this strategy has a number of serious drawbacks that limit a broader applicability. First, since one of the reactants is always the RNA itself, only catalysts for modification reactions of RNA (or chemically modified RNA) can be selected. The majority of reactions of interest to the chemist, namely bimolecular reaction between two small reactants, can not be accessed. Second, this approach selects primarily for substrate properties of the RNA molecules. Library members being excellent catalysts but poor substrates do not acquire the anchor group and are therefore eliminated in the selection step.

To overcome these limitations, the Eaton group and our lab developed an approach involving linker-coupled reactants.[50-53]

4.4 Direct Selection with Linker-Coupled Reactants

To select RNA catalysts for a general reaction A+B → A-B, the potential reactant A is attached to each molecule of a combinatorial RNA library via a long flexible polymeric tether (preferably polyethylene glycol). This library of RNA-linker-substrate conjugates is then incubated with reactant B which carries the anchor group. If an RNA molecule catalyzes the reaction of the attached substrate A with B, it becomes linked to the anchor group and can be selected and amplified (Figure 5).

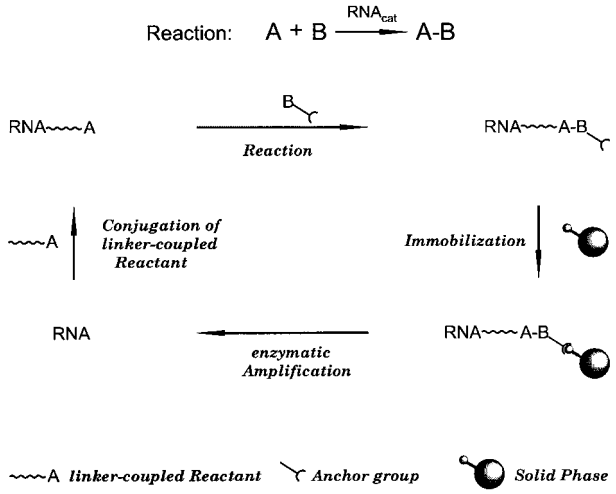

Figure 5. Direct selection of catalytic RNA with linker-coupled reactants.

Eaton and co-workers [50,51] combined this technique with the use of chemically modified nucleotides that contained additional functional groups. Imidazolyl- and pyridyl moieties, respectively, were attached to the C-5 position of all uridines in their RNA libraries, to provide for additional hydrophobic and dipolar interactions, hydrogen bonds and metal ion coordination.[11] From such modified RNA libraries they identified molecules that accelerated a Diels-Alder reaction about 800-fold,[50] and amide synthases that catalyzed the reaction of a tethered primary amine with an activated anhydride about 10^5-fold.[51]

Our lab used direct selection with linker-coupled reactants to isolate ribozymes that catalyze a Diels-Alder reaction of linker-coupled anthracene and biotinylated maleimide (Figure 6a).[53] We generated a library of $2 * 10^{14}$ different RNA-PEG-anthracene conjugates, and allowed them to react with biotin maleimide. Biotinylated reaction products were isolated using a streptavidin matrix and amplified. After 10 rounds of conjugation, reaction, selection, and amplification, the reactivity of the enriched pool was increased about 10^4-fold. We could identify several different sequence families, the best of which accelerated the reaction about 20,000-fold. We identified a small motif present in 90% of all active sequences (Figure 6b). Although we could not detect an accelerated reaction towards free anthracene, we could rationally convert this

motif into a true catalyst that accelerates the Diels-Alder reaction of short anthracene-oligonucleotides about 15,000-fold with multiple turnover.[53]

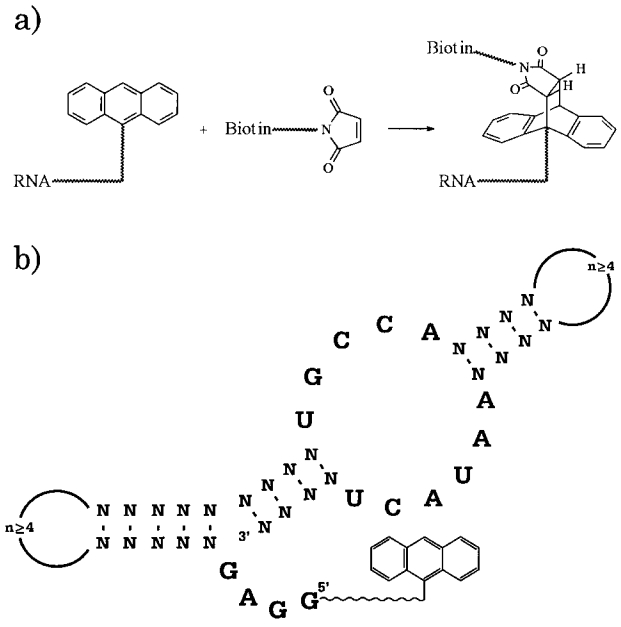

Figure 6: a) Diels-Alder reaction of linker-coupled anthracene and biotin maleimide; b) Proposed secondary structure motif of the selected Diels-Alderase ribozymes.

These examples demonstrate that the catalytic repertoire of nucleic acids is by no means limited to few modifying reactions at nucleic acid components. Further progress in the expansion of DNA/RNA catalysis will depend on the development of suitable selection systems that allow the isolation of molecules with exactly the properties one is looking for.

5 In Vitro Selected Nucleic Acids in Organic Synthesis

The following points make it worthwhile to seriously consider the practical use of in vitro selected nucleic acids in organic synthesis:

– DNA/RNA can specifically bind to a wide variety of different targets, and can differentiate between very similar target molecules.

– DNA/RNA can catalyze a wide variety of chemical reactions.

– To identify/develop DNA/RNA species with certain properties, iterative screening procedures can be applied to combinatorial libraries, and the outcome can be controlled by purposely emphasizing or de-emphasizing individual parameters.

– The catalytic and/or binding repertoire of nucleic acids can be expanded by using chemically modified nucleotides.

For a broader practical appreciation, a number of currently persisting obstacles need to be overcome. The organic chemist is used to work in organic solvents. Multi-step synthesis involves the use of protecting groups, and the majority of protected synthons are hydrophobic and insoluble in water. At present, it appears that unmodified RNA is not as efficient in handling hydrophobic targets as it is hydrophilic ones. In fact, examples of selections for this type of molecule are rare. The selection of aptamers against the hydrophobic amino acid valine demonstrated that RNA is capable of solving such hydrophobic binding problems,[15] although with a much lower affinity, compared to other targets of similar size (Table 1). To make RNA compatible with organic building blocks and solvents, several possible solutions have been proposed. Selections could be carried out in aqueous mixtures of (water-miscible) solvents to select those RNAs that form suitable structures also in the presence of solvents. RNA counterions (most commonly Na^+, K^+, Mg^{2+}) might be exchanged against tetraalkylammonium or similar ions. The use of derivatized nucleotides or post-synthetic modification to introduce hydrophobic side chains into the RNA should not only increase the solubility in organic solvents but also provide for better interactions with hydrophobic targets.[11,54]

The discrimination between closely related small organic molecules achieved by relatively small RNA molecules rivals or even exceeds the performance of known antibodies. This differentiation, combined with the ability to rationally emphasize or de-emphasize positions in a given set of targets makes these aptamers potentially interesting for preparative separations, both of synthetic mixtures and of natural product extracts. Immobilized aptamers could serve as tools for difficult chromatographic separation problems especially for the resolution of chiral compounds. The final purification of natural compounds or its key intermediates are of particular interest. Furthermore, the development of purification schemes based on the specific recognition of widely used protecting groups can be imagined to have significant synthetic impact.

Organic molecules complexed with aptamers might have different reactivities towards conventional reagents leading to otherwise inaccessible products. The same should be valid if the reactive reagents themselves are complexed with oligonucleotides. We therefore propose the use of aptamers as chemo- and/or stereoselective ligands in organic synthesis.

The tailoring of catalysts for chemical reactions has always been the chemist's dream. The described examples show that this is actually possible for oligonucleotide-based catalysts. Techniques like counter-selection and in vitro evolution allow to fine-tune and optimize the properties of the enriched catalysts, and with the beginning development of more sophisticated selection strategies, current limitations could be overcome quite soon. At present, the reaction kinetics of most in vitro selected ribozymes are quite sluggish, and conversion of two free reactants with multiple turnover still represents a major

problem. These problems, however, have nothing to do with limited catalytic abilities of RNA/DNA, they are just related to imperfect selection methodology. E.g., Figure 5 shows that multiple turnover is simply not in the selection query. In order to be selected, the RNA molecule just needs to accelerate the reaction once, so it is not surprising that many selected ribozymes exhibit slow – if any – turnover. New selection methodologies need to be developed that address the issues relevant to the organic chemist.

We anticipate that the excellent enantioselectivity in substrate recognition by nucleic acids will finally allow their use as enantioselective catalysts in organic synthesis, as demonstrated recently for catalytic antibodies. Sinha et al. used an antibody to catalyze the formation of chiral β-hydroxyketones, which then were used to elaborate the complex natural product epothilone.[39] Similarly, acceleration of disfavored chemical reaction pathways is also an attractive target, which might significantly shorten some multistep organic syntheses. E.g., antibodies were generated that accelerated the kinetically highly disfavored exo-Diels-Alder reactions and anti-Baldwin cyclizations. It is conceivable to develop DNA/RNA catalysts with similar properties, thereby allowing one-step syntheses of compounds that would otherwise require high synthetic effort.

The application of in vitro selected nucleic acids in organic synthesis will benefit from the technological developments and corresponding cost reductions made with respect to the use of nucleic acid as drugs. While 10 years ago, a DNA synthesis at a 100 mg scale was a costly endeavor, therapeutically relevant DNA oligonucleotides can now be synthesized at a kilogram scale, and with the recent approval of the first oligonucleotide drug, ton-scale production is targeted.[55,56] RNA, too, is already produced in very large scale, making in vitro selected oligonucleotides available to the organic chemist.

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