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Novel DNA Catalysts Based on G-Quadruplex Recognition

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Recently several approaches have been developed to exploit the inherently selective duplex formation of complementary DNA strands to promote chemical reactions at concentrations that usually obviate efficient catalysis.[1] DNA-templated synthesis is based on bringing small-molecule reactants in close proximity through DNA conjugation and hybridization, and thereby increasing the effective molarity, which significantly accelerates rates of chemical reactions. Several applications of this approach, including compound discovery from synthetic libraries, reaction discovery, and nucleic-acid sensing, have been described.[1] All of the depicted approaches have in common the fact that the two reactants or a reactant and a catalyst are covalently linked to two DNA or PNA strands and are subsequently brought to react by self hybridization or aligned through hybridization. Roelfes and Feringa reported a Diels–Alder reaction mediated by a catalyst intercalated into a DNA duplex. Binding to the duplex resulted in good enantioselectivity of the reaction.[2] Poulin-Kerstien and Dervan employed binding of two polyamides to double-stranded DNA (dsDNA) to promote a 1,3-dipolar cycloaddition; this resulted in linked polyamides.[2] Previously, we found that proline-modified DNA acts as a catalyst in the aldol reaction between a complementary DNA-tethered aldehyde and various nontethered ketones.[3] The formation of a Watson–Crick duplex between the complementary DNA strands was essential for high catalytic efficiency. Here, we show that small-molecule recognition that is selective for a DNA secondary structure, instead of hybridization, can be exploited to promote catalysis between two reactants that are not tethered to DNA.

Guanine-rich DNA sequences are prone to folding into tetraplex structures. Small molecules that bind to such G quadruplexes have recently received great attention since these nucleic acid motifs seem to represent valuable pharmaceutical targets. For example, the telomeres at the end of our chromosomes are composed of G-rich repeats that are able to fold into quadruplex structures.[4] In addition, potential quadruplex-forming sequences have been found to be enriched in promoters of proto-oncogenes.[5] Interestingly, a variety of small molecules that are known to bind to G tetraplexes have displayed antitumor activity.[6] Probably the best characterized compounds are cationic porphyrins, in particular the tetramethylpyridinium porphyrin TMPyP4.[7,8] Since it has been shown that TMPyP4 is able to bind to different types of G quadruplexes we wondered whether we could exploit the small molecule–quadruplex interaction to catalyze a DNA-templated reaction that does not need the hybridization of two or more DNA strands, with nontethered reactants. As proline[9] and prolinamide[10] have shown the extraordinary ability to catalyze aldol reactions even in aqueous phase,[11] we wondered whether the covalent attachment of a proline modification to a G-quadruplex DNA at an appropriate position would enable catalysis of an aldol reaction between a ketone and a porphyrin-tethered aldehyde (Scheme 1).

In order to test our hypothesis, we chose the 15-mer DNA sequence of a thrombin-binding aptamer, d(GGTTGGTGTGGTTGG), which is known to fold into an antiparallel, chair-like G-quadruplex structure in potassium-containing buffer solutions.[12]

The porphyrin-tethered aldehyde **1** was easily synthesized by starting from tris(4-pyridyl)-(4-aminophenyl)porphyrin,[13] which was then conjugated to an aldehyde group by amide bond formation (see the Supporting Information for details). CD spectroscopy confirmed the presence of an antiparallel quadruplex in the presence of porphyrin-tethered aldehyde **1** (Supporting Information). The occurrence of an intense negative peak at 421 nm and a positive peak at 445 nm upon addition of the aldehyde-containing porphyrin indicates a strong interaction between the porphyrin and G quadruplex, due to an induced CD signal from **1**.[14] Having established the binding of the aldehyde-modified porphyrin to the G quadruplex (for detailed binding studies see the Supporting Information), we attached a proline moiety—as a catalytically active functional group—to the DNA. Since literature about the exact binding mode of cationic porphyrins to the antiparallel quadruplex DNA used here was not available, several positions for the attachment of the catalytically active proline were tested. Proline was tethered to multiple positions of the G quadruplex by using commercially available amino modifiers and standard solid-phase DNA synthesis, as described before.[3] Next, we tested the catalytic ability of the proline-modified G quadruplexes for the ability to catalyze the reaction between acetone and aldehyde **1** in phosphate buffer (pH 7.2) containing KCl (100 mM). The reactions were analyzed by HPLC as described in the Supporting Information.[15]

Much to our delight we found that most of the differently modified G quadruplexes (Scheme 2) were able to catalyze the aldol reaction between acetone and **1** at very low concentrations (each 2 μM; Table 1, entries 2–8); product formation was not detected when an unmodified G quadruplex was used (Table 1, entry 1). This shows that the tethered prolinamide was an essential functional group for promoting the aldol reaction. Interestingly, G quadruplexes that were modified on "top" with proline showed relatively poor catalytic efficiency (Table 1,

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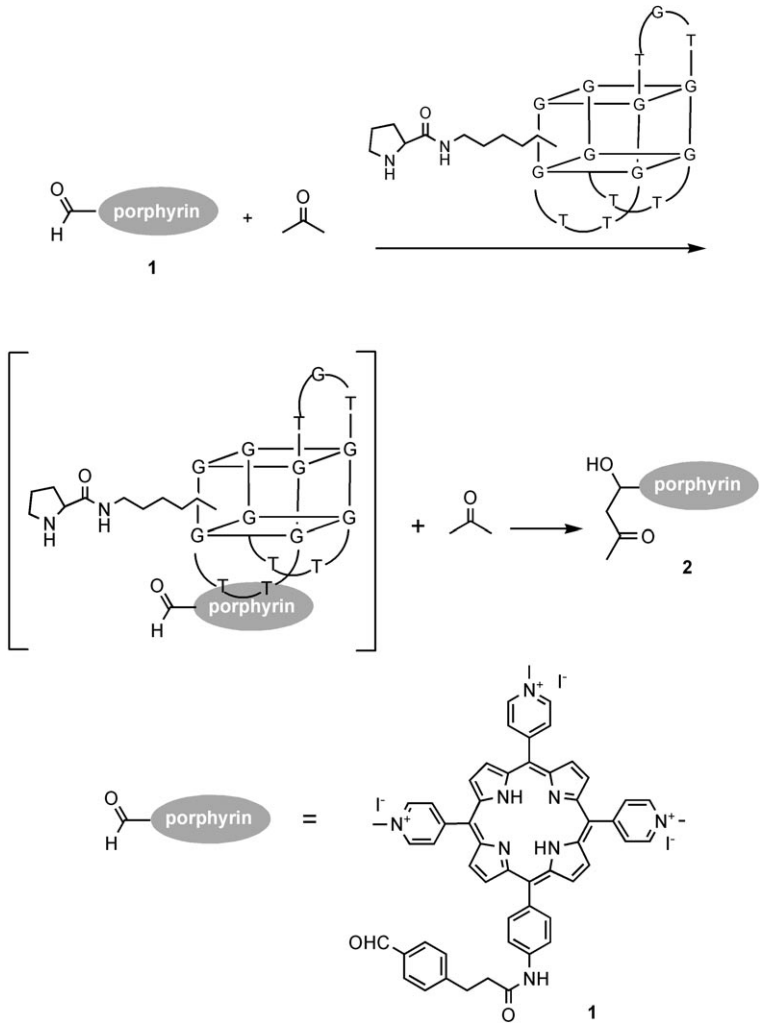
Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.



Scheme 1. DNA catalyst designed for the aldol reaction based on the binding between a G quadruplex and a porphyrin-tethered aldehyde.

Table 1. Aldol reaction catalyzed by different catalysts.

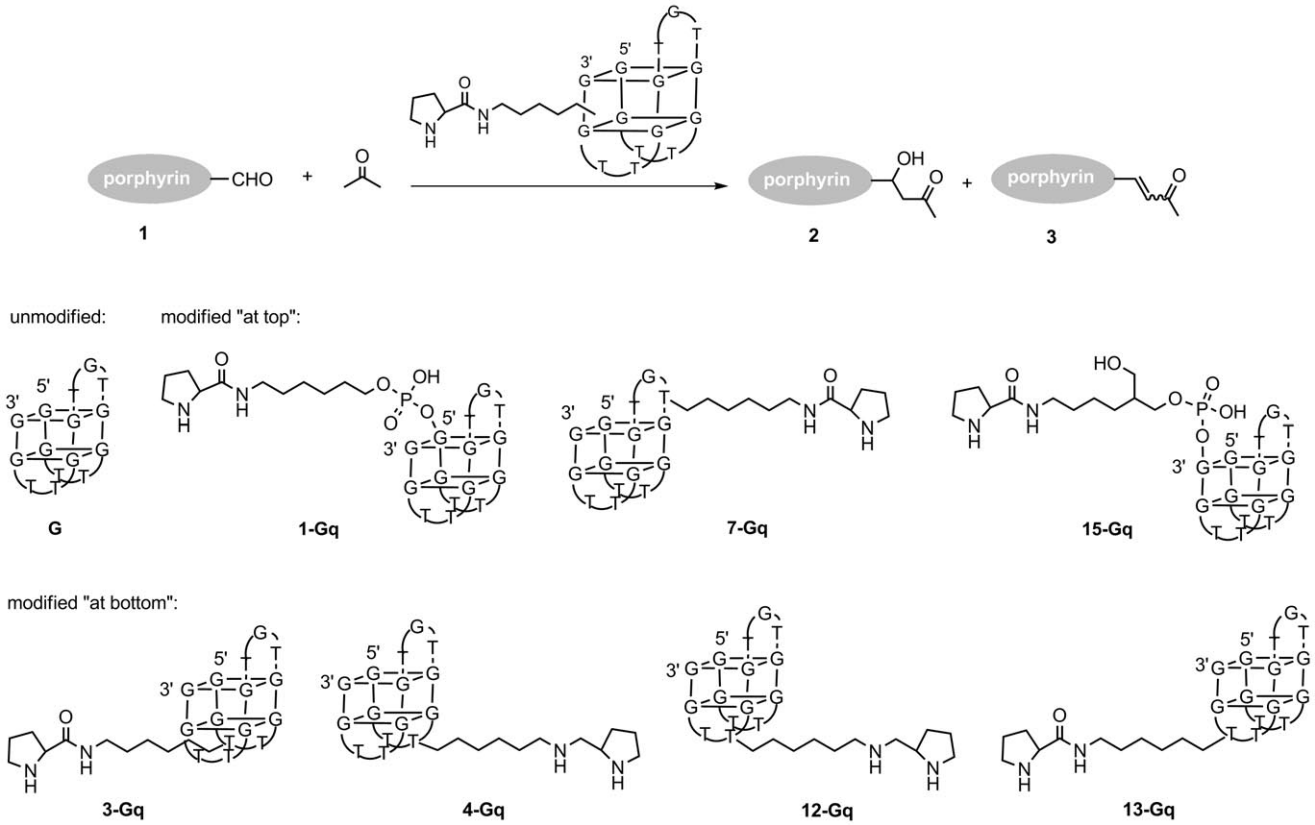
	Catalyst[a]	Yield [%]		
		2	**3**	**2+3**
1	**Gq**	b.d.	b.d.	b.d.
2	**1-Gq**	2%	b.d.	2%
3	**15-Gq**	7%	b.d.	7%
4	**7-Gq**	32%	b.d.	32%
5	**4-Gq**	72%	9%	81%
6	**12-Gq**	45%	4%	49%
7	**3-Gq**	40%	4%	44%
8	**13-Gq**	79%	8%	87%
9	proline[b]	2%	b.d.	2%
10	prolinamide[b]	17%	b.d.	17%
11	–	b.d.	b.d.	b.d.

[a] Conditions: the concentration of oligonucleotides and aldehyde-modified porphyrin **1** was each 2 μM in 100 mM KCl and phosphate buffer (pH 7.2), ratio of aqueous phase/acetone was 5:1 (*v*/*v*). The reaction was incubated at 25 °C for 24 h; b.d.: below detection limit (< 2%). [b] Same conditions except that the concentration of catalyst was 2 mM.

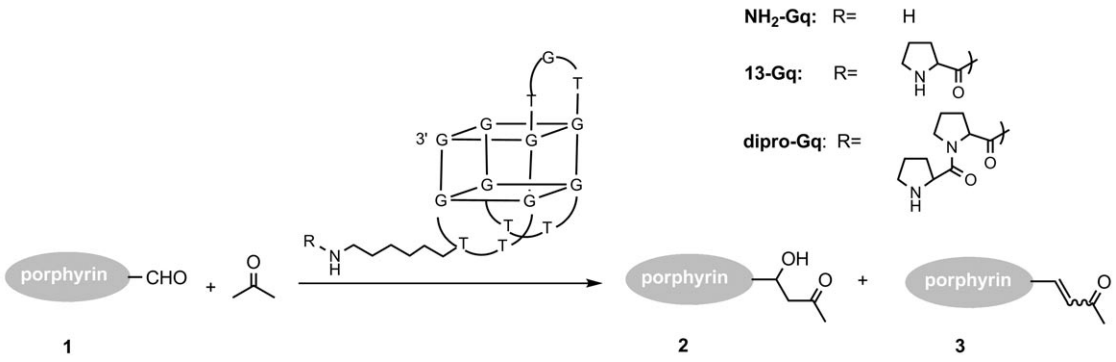
entries 2–4). On the other hand, G quadruplexes with proline tethered to the "bottom" catalyzed the aldol reaction much more efficiently with moderate to high yields (Table 1, entries 5–8). The latter results suggest that the binding position of the porphyrin-tethered aldehyde on the G quadruplex is close to the "bottom" of the G quadruplex (in proximity of the two TT loops). Interestingly, free proline and prolineamide were not able to catalyze the reaction as efficiently as the quadruplex-tethered catalyst even when applied in 1000-fold concentrations (Table 1, entries 9 and 10). Besides achieving the highest yield, the kinetic measurement of initial rate indicated that **13-Gq** is the fastest catalyst compared to G-quadruplex DNA catalysts modified at other positions (Supporting Information). Product was not detected in the absence of catalyst (Table 1, entry 11).

Next, we attempted to optimize the catalytic functional group at the same modification position as in **13-Gq**. A variant of **13-Gq** with a free amine (**NH_2-Gq**, Scheme 3) instead of proline was already available since it served as an intermediate during the synthesis of **13-Gq**. The poor yield of reactions conducted in the presence of **NH_2-Gq** indicates a less efficient catalysis compared to **13-Gq** (Table 2, entry 1). Comparison of the initial rates revealed that the reaction promoted by **13-Gq** ($1.78\ M\ s^{-1}\times10^{-10}$) is about 18 times faster than the one promoted by **NH_2-Gq** ($0.95\ M\ s^{-1}\times10^{-11}$). The initial rate of the same reaction promoted by the small organic catalysts proline ($0.99\ M\ s^{-1}\times10^{-13}$) or prolinamide ($1.34\ M\ s^{-1}\times10^{-13}$) alone is around 1300- and 1800-times slower compared to **13-Gq**. Since it is known that the proline-modified DNA catalyst might form a bicyclic imidazolidinone byproduct through reaction with acetone,[3] we modified the G quadruplex with diproline to get **dipro-Gq** (Scheme 3), which catalyzed the same aldol reaction with moderate yields but without byproduct formation (Table 2, entry 2). Nevertheless, the catalytic efficiency decreased as the initial rate of the reaction promoted by **dipro-Gq** ($3.67\ M\ s^{-1}\times10^{-11}$) was only 20% of the one of **13-Gq**. In the end, **13-Gq** turned out to be the best catalyst for the aldol reaction between acetone and the porphyrin-tethered aldehyde.

The highest yield of aldol product was obtained from the reaction between acetone and aldehyde **1** with the modified G quadruplex catalyst **13-Gq** (Table 2, entry 3). In order to test whether recognition of the four-stranded secondary structure by the porphyrin is necessary for efficient catalysis, a second DNA strand (coDNA), which was complementary to **13-Gq**, was added to the reaction mixture. Hybridization of the C-rich strand with **13-Gq** should result in duplex formation and hence interfere with specific recognition events. The dramatic decrease of the reaction yield as the amount of coDNA was increased from 0.5 to 5 equiv indicated that the quadruplex structure is indispensable for the high catalytic ability (Table 2, entry 4–6). The initial rate of the reaction promoted by **13-Gq**



Scheme 2. Catalysis of aldol reaction by different DNA constructs. The numbers in the names of modified G quadruplexes indicate the position of the modifier on the DNA strand. The different constructs are grouped according to the position of the modification ("at top" or "at bottom" of the quadruplex).



Scheme 3. Optimization of DNA catalysts.

with the same amount of coDNA ($2.38\ M\,s^{-1}\times10^{-12}$) was 75 times lower than the one promoted by **13-Gq**. Therefore, the best catalytic yield by quadruplex catalyst **13-Gq** results from the binding between the G quadruplex and the porphyrin, and depends on the appropriate position of the tethered catalytic group.

So far, the catalyst DNA and porphyrinic aldehyde were treated at a 1:1 stoichiometry. We next investigated whether **13-Gq** was able to promote the reaction in substoichiometric amounts. We indeed found that **13-Gq** could catalyze the aldol reaction using catalytic loading. With 20 mol% of **13-Gq**, the reaction between actone and the porphyrin-tethered aldehyde proceeded to 64% yield after 24 h (Table 2, entry 7). A higher yield of 77% was obtained by increasing the concentration of substrates to 20 μM (Table 2, entry 8). Decrease of the substrate and catalyst concentration below 1 μM resulted in less product formation (data not shown). To gain insights into the catalyst–substrate interactions we determined the binding constants of catalyst **13-Gq** and the porphyrin-tethered aldehyde **1**, as well as that of catalyst **13-Gq** and aldol product **2** using surface plasmon resonance (SPR).[16] Since SPR requires the immobilization of one binding partner, the resulting K_d values represent

Table 2. Aldol reaction catalyzed by different catalysts.

	Catalyst	Yield [%]		
		2	**3**	**2+3**
1	**NH_2-Gq**	32%	b.d.	32%
2	**dipro-Gq**	53%	4%	57%
3	**13-Gq**	79%	8%	87%
4	**13-Gq** + coDNA[a]	64%	4%	68%
5	**13-Gq** + coDNA[b]	14%	b.d.	14%
6	**13-Gq** + coDNA[c]	11%	b.d.	11%
7	**13-Gq**[d]	60%	4%	64%
8	**13-Gq**[e]	71%	6%	77%

[a] 0.5 equiv of coDNA; coDNA: d(CCAACCACACCAACC); [b] 1 equiv of coDNA; [c] 5 equiv of coDNA. [d] The concentration of porphyrin **1** was 2 μM, and catalyst loading was 20%. [e] The concentration of porphyrin **1** was 20 μM, and catalyst loading was 20%.

only estimates. On the other hand, a comparison of the binding of reactant and product of the aldol reaction to the quadruplex is possible. The equilibrium dissociation constants obtained for **1** and **2** from the SPR experiments show that both aldehyde as well as aldol product bind very tightly to G quadruplex **13-Gq** (K_d = 71 nM and 98 nM, respectively). The moderate differences in the equilibrium dissociation constants might explain why **13-Gq** does not achieve high catalytic turnovers.

In summary, we have rationally designed a new DNA catalyst scaffold based on the binding between a G quadruplex and porphyrin. The optimized proline-modified G quadruplex can catalyze the aldol reaction between acetone and porphyrin-tethered aldehyde with high efficiency. More than three orders of magnitude of rate enhancement were achieved by using the quadruplex-based proline catalyst compared to aldol reaction rates observed with small organic catalysts. The presented results indicate the feasibility of tethering nucleic acids with catalytic functionalities to enable artificial catalytic activity towards small molecules bound to the DNA structure. Remarkably, the reaction shows high topological selectivity since dramatic differences that depended on the site of catalyst attachment were found. Hence, the presented strategy could be useful in mapping binding sites of even more complex nucleic acid structures. Here, we have shown that one can exploit a small molecule–DNA interaction for constructing a nucleic acid catalyst. Our finding that a proline-conjugated DNA-secondary structure catalyzes even intermolecular aldol reactions between nontethered reactants broadens the methodological repertoire of DNA-templated reactions, and should be useful for the development of sensors specific for certain nucleic acid structures.

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Keywords: aldol reaction · DNA catalysis · DNA-templated reaction · G quadruplexes · porphyrins

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