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COMMUNICATIONS

Published on Web 09/23/2003

DNA-Templated Metal Catalysis

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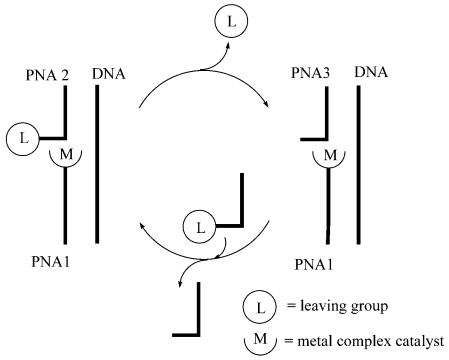
DNA-templated reactions of chemically modified oligonucleotides[1] are attracting much attention for the nonenzymatic detection of nucleic acids,[2] in chemical replication systems,[3] and for the evolution of small molecule libraries.[4] Most reactions of this type involve the covalent ligation of two modified oligonucleotide strands which are complementary to the template. Nonenzymatic DNA ligation with fluorescent reporter probes is among the most sequence selective DNA/RNA sensing strategies because short oligonucleotides exhibit high discrimination of single base mismatches. However, sensitivity is limited by the high affinity of ligated product to the target nucleic acid which prevents efficient signal amplification.[5]

Product inhibition is overcome when ligation is followed by chemical conversion, as exemplified by template-directed imine coupling with subsequent reduction of amine, but the strategy appears to be limited to very short DNA sequences.[3c] When ligation is replaced by a catalytic cleavage reaction, turnover has been observed for DNA-triggered organocatalytic aryl ester cleavage.[6] While very few reactions based on catalysis by functional organic groups proceed smoothly and at an acceptable time scale under the conditions required, a more versatile catalytic concept would facilate the optimization of nucleic acid-templated reactions with signal amplification for specific applications.

Metal catalysis is generally more efficient and more versatile than organocatalysis. Here, we describe a DNA-templated cleavage reaction in which a metal complex − attached to an oligonucleotide analogue − is the catalytic component. In view of our background in copper-catalyzed carboxylic and phosphate ester cleavage,[7] we focused on an ester substrate. Conjugates of copper complexes and oligonucleotides have previously been used for the sequence-specific cleavage of nucleic acids and other applications.[8]

The proposed catalytic cycle is illustrated in Figure 1. Metal complex and substrate (a carboxylic ester with a pyridine-2-carboxylate leaving group L) are attached to short sequences of peptide nucleic acids (PNAs) and brought in proximity at a complementary DNA template. PNA is a nucleic acid analogue in which the sugar phosphate backbone is replaced by a polyamide backbone (Figure 2).[9]

The use of PNA instead of DNA in the present context has the advantages of (a) high target affinity of short oligomers combined with better discrimination of single mismatches[10] and (b) compatibility of carboxylate esters with standard solid-phase PNA synthesis and deprotection conditions. Very few DNA-templated reactions involving oligo-PNA have been reported.[11] In PNA1 (Figure 3), a copper(II) chelating pyridylpyrazolyl group[12] is linked to the α-amino group of a C-terminal lysine. PNA2 and PNA2a, the ester substrates (Figure 3), were prepared by attaching ethylene glycol via a carbamate linker (leading to the alcohol PNA3) or hydroxyacetyl residue via an amide bond (PNA3a) to the N-terminus, followed by esterification with pyridine-2-carboxylic acid. It is well documented that copper(II)-catalyzed hydrolysis of alkyl picolinates is much faster than that of simple esters due to the anchoring effect of the pyridyl donor.[13] Simple alkyl ester analogues of PNA2 are indeed unreactive in the assays described below.



PNA 1 (N-end) Lys-GGATGGA-(ligand) (C-end)
PNA 2 (N-end) (ester)-TCA CAA CTA-Lys_n (C-end) (n=1: PNA2, n=3: PNA2a)
PNA 3 (N-end) (alcohol)-TCA CAA CTA-Lys_n (C-end) (n=1: PNA3, n=3: PNA3a)
DNA 1 5′-TAG TTG TGA TCC ATC C-3′
DNA 2 5′-TA**T** TTG TGA TCC ATC C-3′
DNA 3 5′-TAG TTG TGA TC**T** ATC C-3′

Figure 1. DNA-templated cleavage of ester PNA2 by Cu complex PNA1, catalytic cycle; mismatch bases are underlined.

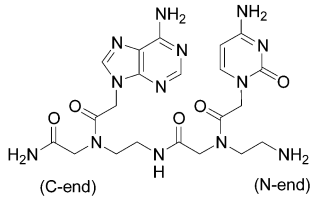


Figure 2. Structure of PNA 2-mer (CA).

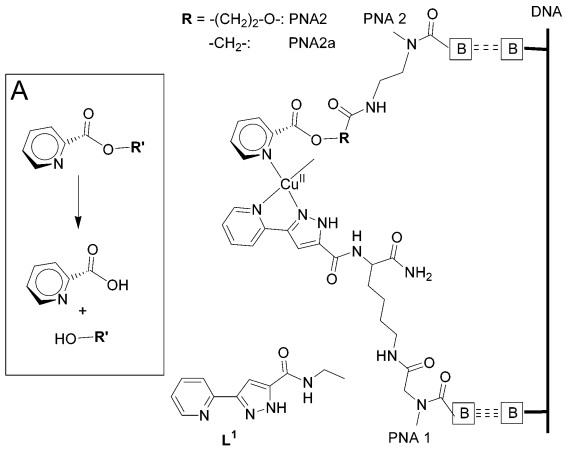


Figure 3. Structure of 3-(pyrid-2-yl)pyrazole and 2-picolinate moieties attached to the C-terminus of PNA1 and N-terminus of PNA2 and PNA2a, respectively. Bottom: Structure of ligand $\mathbf{L^1}$. Inlet A: scheme of picolyl ester hydrolysis.

While it was difficult to investigate the interaction of Cu^{2+} directly with the chelating group of PNA1 under the conditions of

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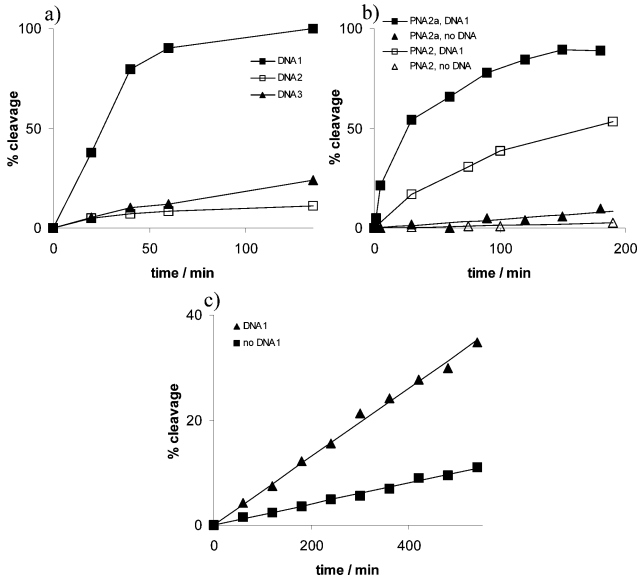

Figure 4. Cleavage of PNA2 and PNA2a by PNA1-Cu^{2+} (water, pH 7, 10 mM MOPS buffer, 50 mM NaCl, $T = 40$ °C): (a) 1 μM PNA1, 1 μM $CuSO_4$, 1 μM PNA2, 1 μM DNA1−3; (b) 1 μM PNA1, 1 μM $CuSO_4$, 5 μM PNA2a (with 1 μM DNA1, ■; without DNA, ▲) or PNA2a (with 1 μM DNA1, □; without DNA, △); (c) 1 μM PNA1, 1 μM $CuSO_4$, 100 μM PNA2a, 1 μM DNA1.

kinetic experiments, we were able to determine by spectrophotometric titrations a 1:1 complexation of Cu^{2+} with the model ligand **L**[1] with involvement of the pyridyl chromophore. The binding constant K is $4(\pm 2) \times 10^7$ M^{-1} at pH 7 and 25 °C. PNA1 and PNA2 were combined with Cu^{2+} sulfate at pH 7 and 40 °C in the presence of DNA1−3 (Figure 4). Hydrolytic cleavage of ester PNA2 was analyzed by HPLC (increase of PNA3 and decrease of PNA2 signal) and confirmed by MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry. Rates of PNA2 and PNA2a cleavage were obtained from linear parts of % cleavage versus time plots at less than 20% ester conversion (Figure 4). At 1 μM PNA1 and equimolar PNA2, Cu^{2+}, and complementary DNA1 in aqueous solution, 38% ester is cleaved after 20 min (Figure 4a, ■), while cleavage is very slow in the absence of DNA (initial rates 150 times smaller, only 3% cleavage after 200 min) or PNA1 or Cu^{2+}. A single mismatch within the PNA1/DNA or PNA2/DNA hybrid (Figure 4a, ▲ □) reduces the initial cleavage rate 7- and 15-fold. This is a consequence of the reduced stability of the mismatched hybrids: melting temperatures T_m[14] are 56.5 °C for PNA1/DNA1 versus 38.1 °C for PNA1/DNA3, and 50.7 °C for PNA2/DNA1 versus 27.1 °C for PNA2/DNA2. When ester PNA2 is used in 5-fold excess (Figure 4b), catalytic conversion is observed with 55% cleavage after 3 h. At 50% cleavage, the reaction rate is about one-half of the initial value, as expected in view of the similar target affinities of ester substrate PNA2 and alcohol product PNA3 ($T_m = 47.7$ °C vs 50.7 °C). At a 100-fold excess of PNA2, the background rate is high, but cleavage is still 1.5-fold faster in the presence of the DNA template. Preliminary studies with PNA2a (Figure 3), in which the picolinate ester is attached by a different linker to the PNA N-terminus, indicate that the problem of high background rate can be reduced by variation of N-terminal PNA modifications. At a 5-fold excess of PNA2a, the initial cleavage rate is 97 times faster than background in the absence of DNA1 (Figure 4b), which corresponds to 485-fold acceleration of ester hydrolysis on the template. This is possibly a consequence of improved preorganization of the ester in PNA2a by the shorter and less flexible glycolic acid linker. At a 100-fold excess of PNA2a, cleavage is still 3.3 times faster in the presence of DNA1. The rate of hydrolysis is practically constant up to at least 35 turnovers (Figure 4c). Rates of background and templated cleavage of picolinate esters of N-modified PNA are not influenced by a number of C-terminally attached lysine residues, as it was shown for PNA2 and its analogue having three lysines on its C-terminus.

Interestingly, released pyridine-2-carboxylate − despite its rather high Cu^{2+} binding constant[15] $K = 10^8$ M^{-1} − does not strongly inhibit the reaction. Possibly a quaternary DNA1/PNA2/PNA1/Cu^{2+} complex is stabilized by coordination of the pyridyl group of PNA2 to Cu^{2+}, as suggested in Figure 3.

In conclusion, the first example of a nucleic acid-templated metal complex catalysis has been reported. The versatility of metal catalysis will facilitate the optimization for specific applications.

Supporting Information Available: Examples of the quantification of PNA2 and PNA3 by MALDI-TOF and HPLC; syntheses of PNA1−3 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0365429