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DNA-templated catalysis using a metal-cleavable linker

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Catalytic release of carboxylates from their 8-hydroxyquinoline esters by Cu(II) complexes, both attached to peptide nucleic acid strands, is triggered by complementary DNA.

DNA-templated ligation of chemically modified oligonucleotides is a promising approach for the non-enzymatic, highly selective detection of DNA and RNA sequences.[1] The sensitivity of the method is limited by the high affinity of ligated product for the target nucleic acid, which prevents efficient signal amplification.[2] Product inhibition is overcome when ligation is replaced by a catalytic cleavage reaction, as recently demonstrated by organocatalytic and metal-catalysed ester cleavage at DNA templates.[3]

However, the reported reactions require rather specific oligonucleotide-conjugated ester substrates having reactive aryl alcohol or carboxylate leaving groups that contain a metal-anchoring donor group. Such limitations restrict the versatility and applicability of DNA-templated catalysis. More general concepts that facilitate probe design for optimal application-dependent performance are highly desirable. Here, we extend our approach of DNA-templated metal catalysis to the release of, in principle, any carboxylate attached to the oligonucleotide by incorporation of an anchoring donor group into a metal-cleavable linker (Scheme 1).

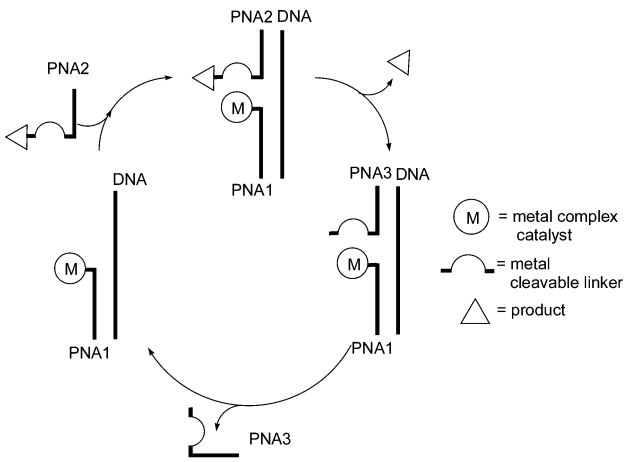
Oligopeptide nucleic acids (PNAs) were used as oligonucleotide analogues due to the better compatibility of standard automated synthesis with the chemical modifications, *i.e.* the ester group.[4]

The synthesis of PNA 1 in which a chelating pyridylpyrazolyl ligand is attached to the C-terminus is described elsewhere.[3c] Ester-modified PNA 2 was prepared as follows: first, the metal-cleavable group was introduced by attaching the unprotected 8-quinoloxy-2-carboxylic acid to the N-terminus of the PNA. This group has been used in peptide chemistry as a metal ion-sensitive protecting group.[5] Then, the intermediate was esterified with benzoic acids. The purity of the products was 30% according to MALDI-TOF (matrix-assisted laser desorption ionisation time-of-flight) mass spectrometry and HPLC analysis. All PNA conjugates were purified by HPLC and fractions containing more than 90% of the desired product were combined, lyophilised and used for further experiments. PNA 1 and PNA 2 were combined with copper(II) sulfate at pH 7 and 40 °C in the presence of DNA 1–3. Hydrolytic cleavage of PNA 2 was analysed using quantitative MALDI-TOF mass spectrometry (increase in PNA 3 and decrease in PNA 2).[6] Rates of PNA 2 cleavage (Scheme 2) were obtained from the linear parts of percentage cleavage *versus* time plots at less than 20% ester conversion. Results were confirmed by quantitative HPLC of the PNA fragments.[7]

At 1 μM PNA 1 and equimolar PNA 2a, Cu(II) and complementary DNA 1, 24% of the substrate is cleaved after 20 min in 4 : 1 water–acetonitrile, while no cleavage was observed in the absence of DNA 1 (data not shown). To avoid problems related to the poor solubility of PNA 2a in water, we used the *p*-sulfobenzoic acid ester PNA 2b in further experiments.

At 1 μM PNA 1 and equimolar PNA 2b, Cu(II) and complementary DNA 1, 73% of the ester is cleaved after 30 min (Fig. 1), while cleavage is very slow in the absence of DNA (initial rate 146 times lower, only 3.5% cleavage after 120 min) (Fig. 2).

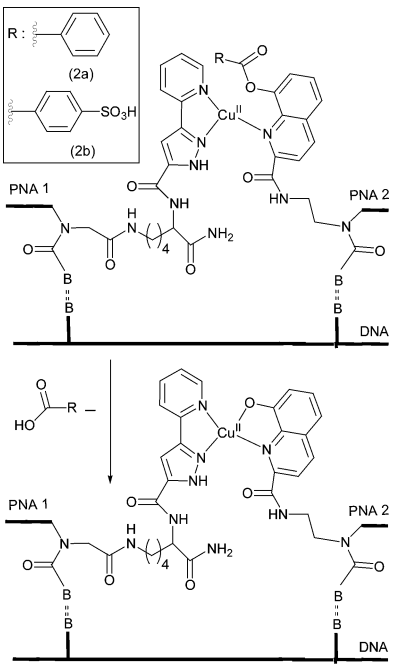
A single mismatch within the PNA 2b/DNA duplex reduces the initial cleavage rate fourfold (data not shown). This is a consequence of the reduced stability of mismatched hybrids.[8]



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$^{3'}$

PNA 1 $^{N'}$(Lys)n-GGATGGA$^{C'}$ (ligand) n=1,3
PNA 2a $^{N'}$(H-benzoic acid ester) TCA CAA CTA-Lys$^{C'}$
PNA 2b $^{N'}$(HSO_3-benzoic acid ester)TCA CAA CTA-Lys$^{C'}$
PNA 3 $^{N'}$(HO-quinoline)TCA CAA CTA-Lys$^{C'}$

Scheme 1 Catalytic hydrolysis of ester–PNA 2 by Cu(II)–PNA 1 triggered by a DNA template.



Scheme 2 Structure of 3-(pyrid-2-yl)pyrazolyl and benzoic acid moieties attached to the C-terminus of PNA 1 and the N-terminus of PNA 2.

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At a hundredfold excess of substrate PNA 2b, the background rate is high, but ester cleavage is still 2.1 times faster in the presence of the DNA template (Fig. 3).

The reaction proceeds with approximately 10 turnovers. Product inhibition is observed, but is less significant than expected in view of the high Cu(II) affinity of the 8-hydroxyquinoline moiety.[9]

In summary, the use of modified oligonucleotides with metal-cleavable linkers in DNA-templated catalysis improves the flexibility of probe design for nucleic acid detection with signal amplification. Cu(II) also promotes the release of amines from *o*-carbamoyl-8-hydroxyquinoline.[5] Synthesis of the carbamoyl analogue of PNA 2 (R = NHR′) is under investigation.

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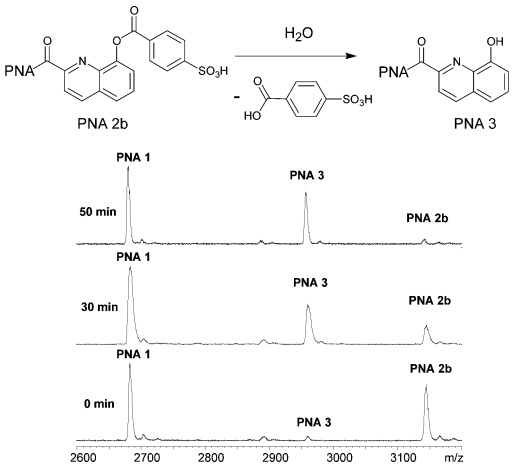

Fig. 1 Reaction scheme and MALDI-TOF spectra of reaction solutions containing PNA 2b and Cu(II)–PNA 1 in the presence of complementary DNA 1 after 0, 30 and 50 min (water, pH 7, 10 mM MOPS buffer, 50 mM NaCl, 40 °C): 1 μM PNA 1, 1 μM PNA 2b, 1 μM Cu(II), 1 μM DNA 1.

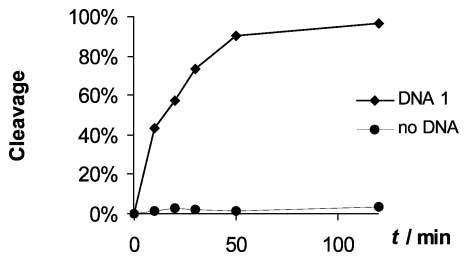

Fig. 2 Cleavage of PNA 2b by Cu(II)–PNA 1 in the presence and absence of complementary DNA 1 (water, pH 7, 10 mM MOPS buffer, 50 mM NaCl, 40 °C): 1 μM PNA 1, 1 μM PNA 2b, 1 μM Cu(II), 1 μM DNA 1.

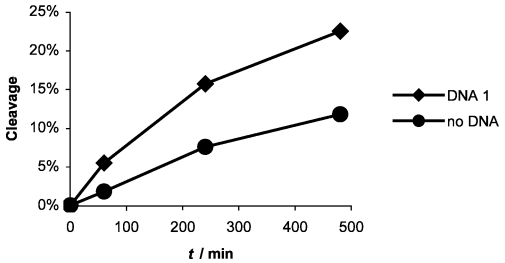

Fig. 3 Cleavage of PNA 2b by Cu(II)–PNA 1 in the presence and absence of complementary DNA 1 (water, pH 7, 10 mM MOPS buffer, 50 mM NaCl, 40 °C): 0.1 μM PNA 1, 10 μM PNA 2b, 0.1 μM Cu(II), 0.1 μM DNA 1.

Notes and references

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7 HPLC was performed as described in ref. 3(*c*). PNA 1: R_t = 23.8 min; PNA 2b: R_t = 29.2 min; PNA 3: R_t = 28.1 min.

8 Melting point of PNA–DNA at 10 mM MOPS, pH 7, 50 mM NaCl, [PNA] = [DNA] = 2 μM: T_m(PNA 2b–DNA 1) = 54.2 °C; T_m(PNA 2b–DNA 2) = 29.0 °C. ΔT_m = ± 2 °C.

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