Metal complex catalysis on a double stranded DNA template

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The reaction of ester hydrolysis catalysed by a DNA duplex in a sequence specific fashion has been developed, which is the fastest and most high yielding in comparison with the known reactions of this type.

Many chemical reactions taking place on single stranded DNA or RNA templates have been described. They include phosphothioate alkylation, formation of phosphodiester, pyrophosphate, amide and disulfide bonds, metal-templated reactions, photochemical 2 × 2 additions and metal- or imidazole-catalysed ester cleavage.[1] These reactions have been used in the detection of single stranded nucleic acids both *in vitro* and *in vivo*.[2]

In contrast to ssDNA, applications of DNA duplexes as templates have been limited. In particular, only stoichiometric ligations on dsDNA have been described, which are rather slow, low yielding and require highly reactive species.[3] DNA is found in a single stranded form in some viruses, while all other living organisms contain double stranded DNA.[4] Application of templated reactions in the direct analysis of specific (*e.g.* repetitive) sequences of dsDNA would be an interesting perspective. This can provide a useful *in vitro* test to find DNA sequences, which are available for binding *in vivo*. Such information can assist in the design of gene specific binders, which are of substantial importance for medicine and biological research. Currently applied methods require denaturation of DNA, which distorts the natural DNA structure.[5] Moreover, the analysis is based on binding of long probes to dsDNA. Two shorter probes are used in the templated reactions, which substantially improves their sequence specificity.[1]

Herein we report on the cleavage reaction, which is triggered in a sequence specific fashion by catalytic amounts of a model double stranded DNA. In this reaction an *N*-methyl-2-imidazole carboxylic acid ester derivative (ester–PNA) is hydrolysed in the presence of a Cu^{2+}-complex–peptide nucleic acid conjugate (PNA–LCu). Both ester–PNA and PNA–LCu bind neighbouring sites on the template, which brings the reacting groups (ester and LCu) in proximity to each other and accelerates the hydrolysis of the substrate ester. PNA is a DNA analogue, which binds polypyrimidine/polypurine DNA duplexes in a sequence specific fashion forming unique polystranded structures of PNA_2–dsDNA stoichiometry (Fig. 1).[6]

Recognition of dsDNA by PNA is not restricted to these sequences. In particular, standard PNA binds to purine rich terminal dsDNA,[7] while so called pseudocomplementary PNAs can target dsDNA of almost any internal sequence.[8]

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Ester–PNA1, PNA1–L (10-mers) and ester–PNA2, PNA2–L conjugates (14-mers) have been synthesised in accordance with protocols described earlier (Scheme 1).[9] Spontaneous hydrolysis of ester–PNAs is rather quick. Therefore, we have used freshly HPLC-purified samples in all kinetic experiments (>80% purity).† The ester is slightly activated by PNA1–LCu complex (18% of ester–PNA cleavage in 180 min, Fig. 2). In the presence of the complementary dsDNA (30-mer DNA **1**–DNA **4**, T_m = 65.0 ± 0.2 °C) the rate of ester–PNA1 hydrolysis is substantially increased (55% after 180 min of the reaction). The template accelerates the initial hydrolysis rate by a factor of 7 (Table 1). Two other dsDNAs have been tested as templates in this reaction. DNA **2**–DNA **6** contained a single mismatch at a binding site of ester–PNA1 and DNA **3**–DNA **5** contained a single mismatch at a binding site of PNA1–LCu. Both of these templates do not significantly affect hydrolysis of ester–PNA1 (entries 2 and 3, Table 1). Polypurine ssDNA **1** accelerates the ester hydrolysis almost as efficiently as DNA **1**–DNA **4** duplex, while polypyrimidine DNA **2** predictably does not affect the hydrolysis rate. This may indicate that similar transition states are formed in the presence of single stranded DNA **1** and the dsDNA. It is well documented that polypurine ssDNAs form PNA_2–DNA associates with polypyrimidine PNAs, which are analogous to those formed by dsDNAs (Fig. 1).[10]

In the presence of catalytic amounts of DNA **1**–DNA **4** duplex (0.2 equiv.) hydrolysis of ester–PNA is still 4 times quicker than the background hydrolysis rate. At these conditions 155% of ester–PNA (relative to the dsDNA) is hydrolysed during 180 min of the reaction.

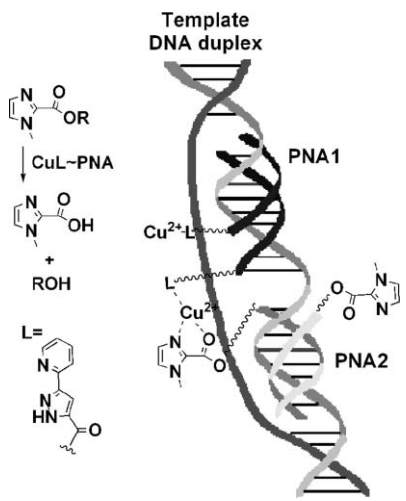
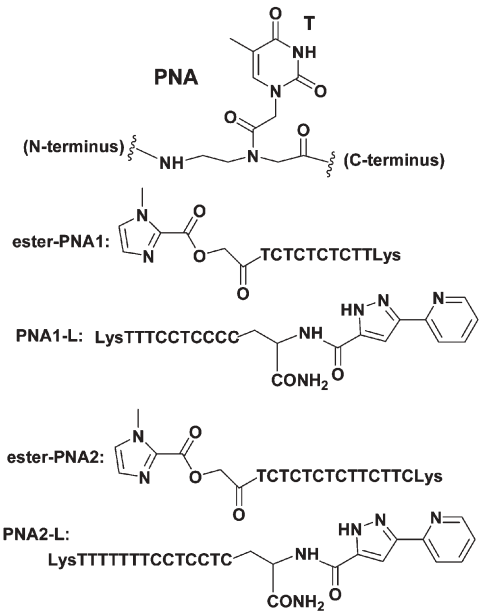


Fig. 1 Cu^{2+} complex (CuL–PNA) catalysed hydrolysis of ester–PNA on a double stranded DNA template. Insert: R is a PNA part of ester–PNA.



DNA1: CGA CGA AGA GAG AGA GGG GAG GAA AGT AGG
DNA2: CGA CGA AGA GA*T* AGA GGG GAG GAA AGT AGG
DNA3: CGA CGA AGA GAG AGA GGG *T*AG GAA AGT AGG
DNA4: CCT ACT TTC CTC CCC TCT CTC TCT TCG TCG
DNA5: CCT ACT TTC CT*A* CCC TCT CTC TCT TCG TCG
DNA6: CCT ACT TTC CTC CCC TCT *A*TC TCT TCG TCG
DNA7: GAG GCT CAC ACA GTA GTT GTT GTC GTT CAA CTT AAC TGC TTC CAA TGA TGT TTT TTT CCT CCT CTC TCT CTC TTC TTC ATT GTA TGC AAT GAT GAG TTG TTG ATG CAC CAC CAG CAC T
DNA8: AGT GCT GGT GGT GCA TCA ACA ACT CAT CAT TGC ATA CAA TGA AGA AGA GAG AGA GAG GAG GAA AAA AAC ATC ATT GGA AGC AGT TAA GTT GAA CGA CAA CAA CTA CTG TGT GAG CCT C

Scheme 1 Sequences of ester–PNA1, PNA1–L, ester–PNA2, PNA2–L and DNAs used in the templated reactions. PNA sequences are written from N- to C-terminus, DNA sequences from 5′ to 3′-terminus. In DNAs **2**, **3**, **5** and **6** mismatched (relative to the corresponding PNA sequences) nucleobases are underscored. DNAs **1–6** are 30-mers and DNAs **7**, **8** are 118-mers.

The dsDNA-templated ester hydrolysis may be potentially inhibited by additional Cu^{2+} binding ligands, which compete with the ester substrate for free sites on the metal ion. This could become a significant limitation in the detection of nucleic acids directly in biological samples, which contain phosphate and chloride anions. The latter can inhibit catalytic activity of Cu^{2+} when present at high concentrations. We have found that ester hydrolysis on double stranded DNA templates does work in a physiological buffer (entries 7–9, Table 1). This may be attributed to exceptionally high stability of the ester–CuL complex, which may be a result of the pre-organization of the corresponding ligands on the dsDNA template (Fig. 1).

The number of catalytic turnovers, which could be achieved in the presence of the catalytic amount of the dsDNA template is rather low (1.55). For example, more than 30 catalytic turnovers have been observed in the related reaction triggered by an ssDNA template, which binds PNAs with formation of the duplex

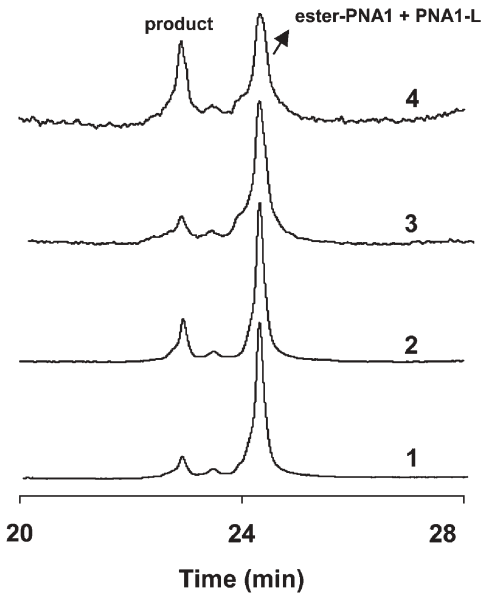


Fig. 2 Monitoring hydrolysis of ester–PNA1 by HPLC. Peaks of ester–PNA1 and PNA1–L conjugates fully overlap with each other under our experimental conditions (R_t = 23–24 min). HPLC trace 1: ester–PNA1 (1 μM), PNA1–L (1 μM), $CuSO_4$ (1 μM) acquired immediately after mixing the components; trace 2: the same as 1, acquired 180 min after mixing the components; trace 3: ester–PNA1 (1 μM), PNA1–L (1 μM), $CuSO_4$ (1 μM), DNA **1**:DNA **4** (1 μM) acquired immediately after mixing the components; trace 4: the same as 3, acquired 180 min after mixing the components. Buffer: 3-(*N*-morpholino) propanesulfonic acid (MOPS) 10 mM pH 7, NaCl 50 mM. The reactions were conducted at 37 °C.

structure.[9] This can be explained by the slow exchange rate of PNA in PNA_2–DNA triplex with free PNA. The latter has been confirmed for different PNA sequences[10] and, in our case, it is manifested by irreversible melting behaviour of the triplexes. This is an intrinsic limitation of dsDNA templated catalytic reactions based on PNA substrates. Further studies are necessary to improve the kinetic lability of PNA_2–DNA triplexes.

There is a possibility that 10-mer PNAs substitute the sense DNA in the 30-mer DNA duplex rather than invade the duplex forming the associate shown in Fig. 1. In this case catalysis would occur on a single stranded rather than double stranded DNA template. To fully exclude this possibility we have tested long (118-mer) double stranded DNA **7**–DNA **8** (Scheme 1) as a template. Due to the length of this dsDNA, its stability is expected to be substantially higher than that of PNA–DNA duplexes. In this case the full substitution of one of the DNA strands in the duplex should not happen.[6,7] Ester–PNA2 and PNA2–L (14-mers), which target a 28-mer sequence in the middle of DNA **7**–DNA **8**, have been prepared. The experiments with this dsDNA have been performed under analogous conditions to those with 30-mer DNA **1**–DNA **4** (entry 5, Table 1), except that Triton X-100 has been included in the buffer to minimise unspecific effects. Cleavage of ester–PNA2 in the presence of PNA2–LCu and catalytic amounts of DNA **7**–DNA **8** is 3 times faster than that of ester–PNA1 (entries 5 and 10 in Table 1). This may reflect a higher binding affinity of ester–PNA2 to the DNA due to its longer PNA sequence (14-mer *vs.* 10-mer). A slightly larger number of catalytic

Table 1 Yields and relative rates of templated cleavage of ester–PNAs[a]

Entry	Template (concentration/μM)	$V_{template}/V_{backgr}$[b]	Buffer[c]	Turnover number[d]
Ester–PNA1 + PNA1–L				
1	DNA **1**–DNA **4** (1)	7.1 ± 1.4	A	0.55
2	DNA **2**–DNA **6** (1)	1.4 ± 0.3	A	0.26
3	DNA **3**–DNA **5** (1)	1.5 ± 0.3	A	0.27
4	DNA **1** (1)	5.1 ± 1.0	A	0.47
5	DNA **1**–DNA **4** (0.2)	4.0 ± 2.1	A	1.55
6	No template	1	A	0.18
7	DNA **1**–DNA **4** (1)	8.0 ± 1.5	B	0.63
8	DNA **1** (1)	7.9 ± 1.4	B	0.56
9	No template	1	B	0.23
Ester–PNA2 + PNA2–L				
10	DNA **7**–DNA **8** (0.2)	12.1 ± 1.0	A	1.65
11	DNA **7** (0.2)	0.9 ± 0.4	A	0.24
12	DNA **8** (0.2)	9.5 ± 1.1	A	1.06
13	No template	1	A	0.24

[a] [ester–PNA] = [PNA–L] = [$CuSO_4$] = 1 μM. [b] Ratio of initial rates of ester–PNA cleavage in the presence and absence of DNAs; background cleavage rate for entries 1–5 and 10–13 was determined in buffer A and for entries 7–9 in buffer B. [c] Buffer A: MOPS 10 mM pH 7, NaCl 50 mM; buffer B: phosphate 10 mM pH 7, NaCl 150 mM. [d] Conversion of ester–PNA into alcohol–PNA (relative to the template amount), which was determined 180 min after the beginning of the reaction.

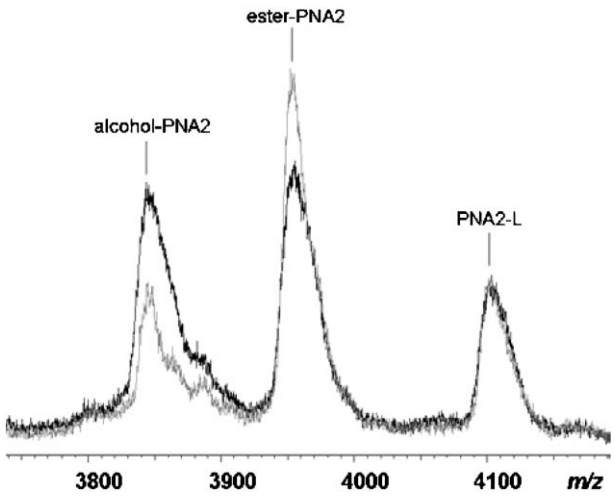


Fig. 3 MALDI-TOF mass spectra of mixtures containing ester–PNA2 (1 μM), PNA2–L (1 μM), $CuSO_4$ (1 μM) (grey trace) and ester–PNA2 (1 μM), PNA2–L (1 μM), $CuSO_4$ (1 μM) with catalytic amounts of DNA **7**–DNA **8** duplex (0.2 μM: 1 μL of this solution was used for MS analysis, which corresponds to 20 fmol dsDNA) (black trace) acquired 180 min after mixing the components. Other conditions: MOPS (10 mM, pH 7), NaCl (50 mM), Triton X-100 (1 mM), temperature 37 °C. The spectra were normalised using the PNA2–L peak.

turnovers has been achieved with the long DNA templates (entries 5 and 10 in Table 1).

MALDI-TOF spectroscopy is a quick and sensitive method of detection of peptide nucleic acids. Combination of this method with the dsDNA templated reaction of ester–PNA hydrolysis can be used in analysis of dsDNAs. We have demonstrated that 20 fmol of 118-mer DNA can be unambiguously detected using MALDI-TOF mass spectrometry (Fig. 3).

A 40% yield of the 30-mer DNA has been obtained after 9 h of the BrCN mediated ligation of 15-mer DNA probes in the presence of a dsDNA template.[3b] In the analogous ligation taking place in the presence of *N*,*N*′-carbodiimidazolyl, 90% of the product was obtained in 48 h.[3a] In comparison with these reactions the process reported here is significantly more efficient: 165% of the product in 3 h. This is the first reaction to be catalysed by double stranded DNAs in a sequence specific fashion.

Notes and references

† Conditions of PNA purification by HPLC: Macherey-Nagel Nucleosil C4 250 × 4.6 mm column with gradients of CH_3CN (0.1% TFA, solvent B) in water (0.1% TFA, solvent A), 49 °C, 0% B for 5 min, in 30 min to 35% B, in 10 min to 90% B, 90% B for 10 min.

Ester–PNA1: HPLC R_t = 23.7 min. Yield 1.8%. MALDI-TOF MS: calcd for $C_{119}H_{158}N_{49}O_{40}$ $[M + H]^+$: 2914.8, found 2914.0. PNA1–L: HPLC R_t = 22.9 min. Yield 2.2%. MALDI-TOF MS: calcd for $C_{125}H_{167}N_{54}O_{37}$ $[M + H]^+$: 3018.0, found 3020.5; ester–PNA2: HPLC R_t = 29.8 min. Yield 4.4%. MALDI-TOF MS: calcd for $C_{161}H_{211}N_{67}O_{65}$ $[M + H]^+$: 3946.6, found 3947.9; PNA2–L: HPLC R_t = 28.3 min. Yield 0.8%. MALDI-TOF MS: calcd for $C_{170}H_{225}N_{69}O_{54}$ $[M + H]^+$: 4096.7, found 4093.5.

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