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Sequence Selective Hydrolysis of Linear DNA Using Conjugates of Zr(IV) Complexes and Peptide Nucleic Acids

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A series of conjugates of peptide nucleic acids (PNA) and Zr(IV) complexes was prepared. Their ability to hydrolyze DNA was tested using MALDI-TOF mass spectrometry and HPLC. The most efficient artificial restriction enzyme found, PNA conjugate with Zr(IV) complex of tris(hydroxymethyl)-aminomethane, cleaves DNA targets sequence selectively in close proximity to the Zr(IV) complex. It was demonstrated that cleavage products are substrates of terminal transferase.

Restriction endonucleases are enzymes which hydrolyze the phosphodiester backbone of deoxynucleic acids (DNA) sequence specifically. High selectivity and efficiency are characteristics of these enzymes, but they recognize only short oligonucleotide sequences, producing many fragments upon cleavage of genomic DNA. Therefore, it is desirable to develop artificial nucleases with higher sequence specificity. Artificial nucleases cleave nucleic acids by either an oxidative or hydrolytic mechanism. The former is observed with redox-active metal complexes;[1] the latter one is based on the Lewis-acidity of metal ions.[2] An important drawback of the oxidative cleavage is the generation of products with unnatural termini, whereas the cleaving products of hydrolytical nucleases can be further processed by enzymes.[1,2] Potential applications as antigene or antisense drugs are predicted.[2,3] Efficient hydrolytic cleavage of linear DNA is only possible with strong lewis acids such as Ce(IV),[4] Zr(IV),[5] Th(IV),[6] and Co(III).[7] Sequence specificity of nucleic acid cleavage by metal complexes is low but can be improved by combination with an oligonucleotide recognition fragment. Most of the reported examples describe the cleavage of RNA, which is more susceptible to hydrolysis than DNA.[2,8] In contrast, very few examples of sequence-selective, nonenzymatic hydrolysis of linear single-stranded DNA have been reported; examples are a chimeric metallopeptide[9] or a conjugate of a Ce(IV) iminodiacetate complex and DNA.[10] Herein we describe the cleavage of linear DNA with conjugates of peptide nucleic acids (PNAs) and complexes of Zr(IV) (Figure 1). PNA[11] is a DNA analogue with a nonnatural polyamide backbone. PNA conjugates have been applied to the sequence specific cleavage of RNA[12] and to redox cleavage of double-stranded DNA[13] but not yet for DNA hydrolysis. Complexes of Zr(IV) catalyze the hydrolysis of activated phosphodiesters and dinucleotides under weakly acidic conditions with an efficiency similar to Ce(IV).[5] Moreover, Zr(IV) is expected to act exclusively as a strong Lewis acid, while Ce(IV) as a redox active ion can be reduced to the less active Lewis acid Ce(III) and catalyze

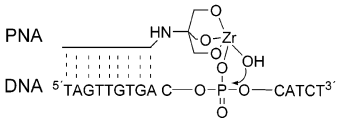

Figure 1. A proposed model for hydrolytic DNA scission by PNA−Zr(IV) conjugate.

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(1) Reviews: (a) Pogozelski, W. K.; Tullis, T. D. *Chem. Rev.* **1998**, *98*, 1089−1108. (b) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1152.
(2) Reviews: (a) Trawick, B. N.; Daniher, A. T.; Bashkin, J. K. *Chem. Rev.* **1998**, *98*, 939−960. (b) Ott, R.; Krämer, R. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 761−767.
(3) (a) Haner, R.; Hall, J. *Antisense Nucleic Acid Drug Dev.* **1997**, *7* (4), 423−430. (b) Matsuda, S.; Ishikuba, A.; Kuzuya, A.; Yashiro, M.; Komiyama, M. *Angew. Chem.* **1998**, *110* (23), 3477−3479.
(4) (a) Sumaoka, J.; Azuma, Y.; Komiyama, M. *Chem. Eur. J.* **1998**, *4*, 205−209. (b) Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L., Jr. *J. Am. Chem. Soc.* **2001**, *123*, 1898−1904. (c) Branum, M. E.; Que, L., Jr. *J. Biol. Inorg. Chem.* **1999**, *4*, 593−600. (d) Franklin, S. J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 201−208.
(5) Ott, R.; Krämer, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1957−1960.
(6) Ihara, T.; Shimura, H.; Ohmori, K.; Tsuji, H.; Takeuchi, J.; Takagi, M. *Chem. Lett.* **1996**, *8*, 687−688.
(7) Dixon, N. E.; Geue, R. J.; Lambert, J. N.; Moghaddas, S.; Pearce, D. A.; Sargeson, A. M. *Chem. Commun.* **1996**, *11*, 1287−1288.
(8) (a) Bashkin, J. K.; Frovola, E. I.; Sampath, U. J. *J. Am. Chem. Soc.* **1994**, *116*, 5981−5982. (b) Magda, D.; Miller, R. A.; Sessler, J. L.; Iverson, B. L. *J. Am. Chem. Soc.* **1994**, *116*, 7439−7440.
(9) (a) Welch, J. T.; Sirish, M.; Lindstrom, K. M.; Franklin, S. M. *Inorg. Chem.* **2001**, *40*, 1982−1984. (b) Kovavic, R. T.; Welch, J. T.; Franklin, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 6656−6662.
(10) Komiyama, M. *J. Biochem.* **1995**, *118*, 665−670.
(11) Nielsen, P. E.; Egholm, M. In *Peptide Nucleic Acids Protocols and Applications*; Nielsen, P. E., Egholm, M., Eds.; Horizon Scientific: United Kingdom, 1999; pp 1−19.
(12) (a) Verheijen, J. C.; Deiman, B. A. L. M.; Yeheskiely, E.; Van der Marel, G. A.; Van Boom, J. H. *Angew. Chem., Int. Ed.* **2000**, *39* (2), 369−372. (b) Whitney, A.; Gavory, G.; Balasubramanian, S. *Chem. Commun.* **2003**, *1*, 36−37.
(13) Bigey, P.; Sönnichsen, S. H.; Meunier, B.; Nielsen, P. E. *Bioconjugate Chem.* **1997**, *8*, 267−270.

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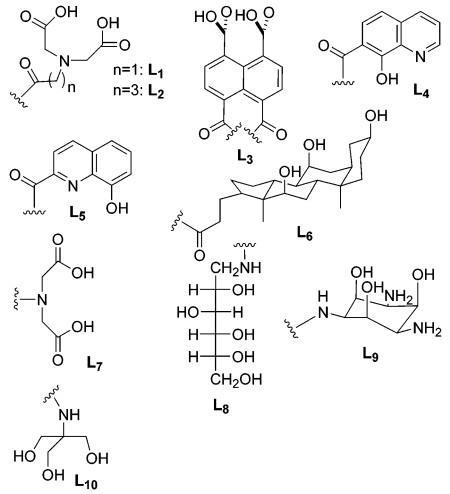
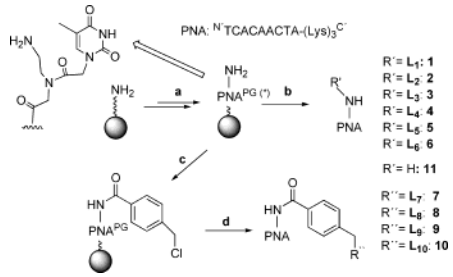
Scheme 1. Modifiers of Termini of PNA



Scheme 2 [a]

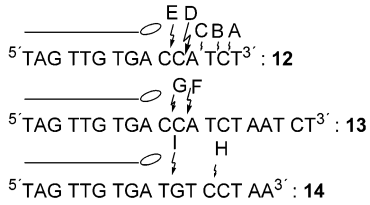


[a] (a) PNA synthesis; (b) (1) R′-OH, HBTU, HOBT, DIEA, DMF; (2) TFA/*m*-cresol; (c) 4-chloromethyl-benzoic acid, HBTU, HOBT, DIEA, DMF; (d) (1) NaI; DMSO, H−R″; (2) TFA/*m*-cresol. PG (*) = Bhoc for nucleobases, Boc for lysine.

side reactions. To avoid complications by self-degradation of a Zr(IV)−DNA conjugate, we use PNA as a recognition motif. We have previously described the synthesis of PNA conjugates with Cu(II), Zn(II), and Ni(II) complexes.[14]

A series of ligands (Scheme 1) was attached to the N-terminus of PNA either by coupling of the commercially available 8-hydroxy-quinoline-2-carboxylic acid (**L$_5$**-H, Scheme 1), protected iminodiacetate derivatives[15] using HBTU activator (Scheme 2),[16] or by coupling 4-chloromethyl-benzoic acid to the N-terminus of the PNA followed by amination of the resulting PNA (Scheme 2).[16] PNAs **3**, **4**, and **6** were synthesized as described elsewhere.[17] Purity of products was 43−86% according to MALDI-TOF mass spectrometry and HPLC analysis. All PNA conjugates were HPLC purified.[18] Fractions containing more than 90% of the desired product were combined, lyophilized, dissolved in deionized water, and used for further experiments. The duplex of nonmodified PNA **11** (Scheme 2) with complementary DNA **12** (Scheme 3) melts at 54.5 ± 0.4 °C. Modifications **L$_1$**−**L$_{10}$** do not affect melting behavior significantly, $\Delta T_m = 0-7$ °C. In accordance with these

Scheme 3. Fragmentation Patterns of DNA **12**−**14** Cleavage by PNA **10** (10 μM)/Zr(IV) (100 μM)



experiments, at 22 °C, pH 7, and micromolar concentrations of PNA and DNA, the PNA−DNA duplex is the only species in solution.

The activity of the Zr(IV) complex−PNA conjugates in the sequence selective cleavage of DNA was analyzed using a combination of MALDI-TOF MS and HPLC. The cleavage experiments were followed by quantitative MALDI-TOF mass spectrometry[19] because of the short time of analysis (less than 1 min) and the small quantities needed (3 pmol for three point assay). The major products of the sequence selective DNA cleavage were quantified for the most active Zr(IV)−PNA conjugate by HPLC.

Sequence selective or random cleavage can diminish DNA **12** concentrations, but only in the former case, are characteristic DNA fragments observable.

Figure 2 shows mass spectra of DNA **12**/Zr(IV) (10-fold excess) mixtures (I−IV) taken 120 h after reaction beginning. The same amount of internal standard (IS) was added to every sample. In all samples containing PNA conjugates, three characteristic peaks (*) were observed for the PNA, a PNA-MOPS and a PNA-matrix adduct. At 1 equiv of Zr-(IV), the activity of PNA conjugate is rather low, indicating incomplete complex formation with Zr(IV). In the absence of any PNA, DNA **12** concentration is reduced by 69 ± 16% 164 h after starting reaction (Figure 3, trace I), but no characteristic fragments of DNA **12** were observed in the MALDI mass spectra (Figure 2, trace I). Therefore, we believe that free Zr(IV) randomly hydrolyzes DNA **12**. Interestingly, this effect is supressed in the presence of unmodified PNA **11** (Figure 2, trace II, and Figure 3, trace II) or unmodified PNA **11** in combination with the external ligand TRIS (**L$_{10}$**-H) (Figure 2, trace III, and Figure 3, trace III). While the modified PNA−Zr(IV) complexes **3**−**6** do

(14) Mokhir, A.; Stiebing, R.; Krämer, R. *Bioorg. Med. Chem. Lett.* **2003**, *13* (8), 1399−1401.
(15) Synthesis of bis(*N-tert*-butyloxycarbonylmethyl)-amino-acetic acid (**17**) and 4-bis(*N-tert*-butyloxycarbonylmethyl)-amino-butyric acid (**18**) is described in the Supporting Information.
(16) Supporting Information.
(17) Mokhir, A.; Zohm, B.; Fuessl, A.; Krämer, R. *Bioorg. Med. Chem. Lett.* **2003**, *13* (15), 2489−2492.
(18) (a) HPLC purification of **1**, **2**, **5**, **7**−**11**. HPLC was on EC 250 × 4.6 mm Nucleosil 300-5 C4. Gradients of 0.1% trifuoroacetic acid (TFA) in CH_3CN (solvent B) in 0.1% TFA in water (solvent A) were used: in 5 min from 0 to 2% B, in 23 min from 2 to 20% B, in 7 min from 20 to 95% B, 10 min at 95% B. (b) Characterization data for **1**, **2**, **5**, **7**−**11**: **1**, yield 49%, HPLC R_t = 29.1 min, MALDI-TOF MS for $C_{120}H_{166}N_{59}O_{33}$ [M + H]$^+$ calcd 2962.6, found 2960.4; **2**, yield 79.9%, HPLC R_t = 27.8 min, MALDI-TOF MS for $C_{122}H_{170}N_{59}O_{33}$ [M + H]$^+$ calcd 2991.0, found 2989.4; **5**, yield 74%, HPLC R_t = 30.4 min, MALDI-TOF MS for $C_{124}H_{164}N_{59}O_{30}$ [M + H]$^+$ calcd 2961.2, found 2961.6; **7**, yield 65%; HPLC R_t = 28.2 min; MALDI-TOF MS for $C_{126}H_{170}N_{59}O_{33}$ [M + H]$^+$ calcd 3039.7, found 3037.7; **8**, yield 79.3%; HPLC R_t = 25.1 min; MALDI-TOF MS for $C_{128}H_{178}N_{59}O_{34}$ [M + H]$^+$ calcd 3087.1, found 3085.1; **9**, yield 42.7%, HPLC R_t = 23.5 min; MALDI-TOF MS for $C_{128}H_{178}N_{61}O_{32}$ [M + H]$^+$ calcd 3083.2, found 3081.4; **10**, yield 75.3%, HPLC R_t = 26.4 min, MALDI-TOF MS for $C_{126}H_{174}N_{59}O_{32}$ [M + H]$^+$ calcd 3027.1 found 3028.0; **11**, yield 85.8%, HPLC R_t = 22.6 min, MALDI-TOF MS for $C_{114}H_{159}N_{58}O_{28}$ [M + H]$^+$ calcd 2789.8, found 2789.2.
(19) Sarracino, D. A.; Richert, C. *Bioorg. Med. Chem. Lett.* **1996**, *6* (21), 2543−2548.

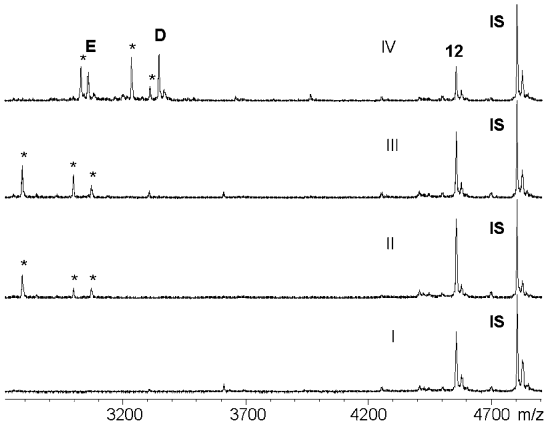


Figure 2. MALDI-TOF spectra 120 h after the start of the reaction. (I) Cleavage of DNA **12** (10 μM) by Zr(IV) (100 μM). (II) Cleavage of **12** (10 μM) by **11** (10 μM)/Zr(IV) (100 μM). (III) Cleavage of **12** (10 μM) by **11** (10 μM)/TRIS (10 μM)/Zr(IV) (100 μM). (IV) Cleavage of **12** (10 μM) by **10** (10 μM)/Zr(IV) (100 μM). Asterisk (*) indicates PNA, PNA−matrix, PNA−buffer. IS = internal DNA standard.

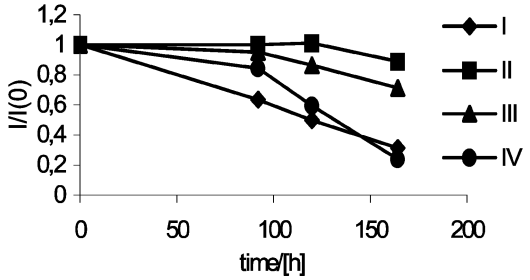


Figure 3. Degradation rate of DNA **12**. I−IV (see Figure 2). Analysis by quantitative MALDI-TOF MS. I: peak intensity, $\Delta I/I(0) = \pm 4-26\%$.

not cleave DNA **12** substantially (data not shown), conjugates of Zr(IV)−PNA with carboxylates (**L_1**, **L_2**, **L_7**) or amino alcohols (**L_8**, **L_9**, **L_{10}**) show DNA cleaving activity (13−76 ± 4−26%; data for **10** is shown in Figure 2, trace IV, and Figure 3, trace IV). In particular, the tris(hydroxymethyl)-aminomethane (TRIS) modified PNA **10** showed 76 ± 19% cleavage of DNA **12** after 164 h from reaction beginning (Figure 3, trace IV) with the desired sequence selectivity (Figure 2, trace IV). This is in agreement with previous studies, where the highest phosphodiester cleaving activity among various Zr(IV) complexes was found for TRIS−Zr-(IV).[5] PNA **10**−Zr(IV) cleaves predominantly those DNA phosphodiester groups, which are in close proximity to the terminal PNA modification (Scheme 3), but excluding the phosphodiester group next to the duplex region. There is no evidence for fragmentation of PNA **10**. The smaller CATCT and ATCT fragments of DNA **12** cleavage are not detectable since they are degraded by free Zr(IV) faster than DNA **12** itself, as shown by control experiments (Figure S18, Supporting Information).

The cleavage occurs between the first and second (fragment E) and the second and third (fragment D) DNA nucleotides of DNA **12**. These results are confirmed by HPLC analysis of the mixture IV (Figure 4). In particular,

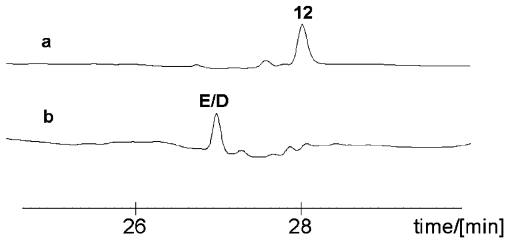


Figure 4. HPLC/MALDI-TOF of (a) cleavage reaction IV of DNA **12** by PNA **10** (10 μM)/Zr(IV) (100 μM) at 0 min and (b) IV after 164 h.

91% of DNA **12** is cleaved after 164 h, and a dominant peak at R_t = 26.7 min was found to contain both fragments E and D. Such behavior was reproduced for two more DNA sequences **13** and **14** (Scheme 2).[16] Both DNAs are cleaved at the second phosphodiester after the duplex region. The fragments are analogous to fragment E of DNA **12** (DNA **13**, cleavage between CC (fragment G); DNA **14**, cleavage between TG (fragment I)). Additionaly, we observed for both DNAs one more fragment (DNA **13**, CA cleavage (fragment F); DNA **14**, CC cleavage (fragment H)). Mismatched DNA is cleaved by Zr(IV)/PNA **10** nonspecifically (data not shown). As it follows from mass measurements, all products of DNA cleavage with Zr(IV)/PNA **10** are natural dephosphorylated DNAs. These DNA fragments were further manipulated using natural enzymes.

For example, we could efficiently phosphorylate cleavage products in the mixture IV (Figure 2, trace IV) by terminal transferase and [α-^{32}P] ddATP.[20]

In summary, conjugates of peptide nucleic acids and Zr-(IV) complexes hydrolyze complementary single-stranded DNA in a sequence selective fashion. Activity is strongly dependent on the nature of chelating ligand. There is significant nonselective background cleavage by excess free Zr(IV) in single stranded DNA regions while DNA is protected within the PNA/DNA duplex. For the optimization of Zr(IV)-based artifical restriction enzymes, chelating ligands that form stronger complexes with Zr(IV) without loss of phosphodiesterase activity are required.

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Supporting Information Available: Experimental details of ligand and PNA synthesis. Description of DNA cleavage reaction and analytical HPLC. MALDI-TOF analysis of cleavage reacions of DNAs **13** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) The 3′-end labeling of **12** was performed under standard conditions using the 3′-end labeling Kit of Roche Diagnostics GmbH. See: http://biochem.roche.com/pack-insert/1028715b.pdf.