The average R value for **3**, **4**, **7**, and **8** is 0.3. See: C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphuis, *J. Am. Chem. Soc.* **1996**, *118*, 2744–2745.

[28] From these data alone we cannot rule out the possibility of a conformational switch from an α- to a 3_{10}-helix (or vice versa) upon ring closure. See: G. Yoder, A. Polese, R. A. G. D. Silva, F. Formaggio, M. Crisma, Q. B. Broxterman, J. Kamphuis, C. Toniolo, T. A. Keiderling, *J. Am. Chem. Soc.* **1997**, *119*, 10278–10285.

[29] Colorless crystals of **8** were grown from CH_2Cl_2/hexane; crystal dimensions $0.3 \times 0.5 \times 0.5$ mm, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 19.30(1)$, $b = 24.73(2)$, $c = 12.134(7)$ Å, $V = 5791(6)$ Å^3, $\rho_{calcd} = 1.246$ g cm^{-3}; $2\theta = 1.6$–$50.0°$ ($Mo_{K\alpha}$ radiation, $\lambda = 0.7107$ Å, graphite monochromator, 2θ-θ scan, $T = 156$ K); 4698 reflections measured, 2266 unique data used [$I > 3.0\sigma(I)$]; no absorption corrections were applied; direct methods (SHELXTL 86), refinement with full-matrix least squares, hydrogen atoms refined isotropically, all other atoms refined anisotropically; $R = 0.066$, $R_W = 0.077$; maximum residual electron density 0.80 e Å^{-3}; GOF = 2.28 for 633 variables. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-101810. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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[32] The 4→1 N ⋯ O=C angles for these four hydrogen bonds range from 118 to 131°.

[33] The analogous hydrogen-bonding pattern in α-helices is 5→1, spanning three residues. See reference [30].

[34] ^1H NMR solvent titration experiments in $CDCl_3/(CD_3)_2SO$ mixtures (5 mM, 25 °C) suggest that **8** may exist as a 3_{10}-helix in $CDCl_3$. Full solution-phase 1D and 2D ^1H NMR analyses of peptides **3**, **4**, **7**, and **8** will be reported in a separate publication.

[35] All peptide helix axes are parallel in the unit cell of **8**. Head-to-tail hydrogen bonding is commonly observed in crystalline hydrophobic peptide helices. See reference [16].

[36] The different medium from which **2** and **8** were crystallized (CH_2Cl_2/hexane for **8**; methanol/H_2O for **2**) may contribute to the disparity in their crystal structures.

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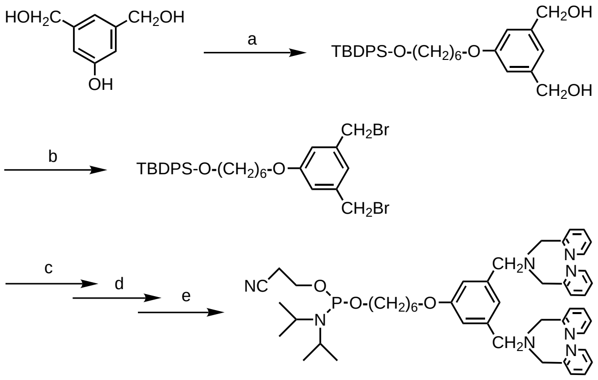
Conjugates of a Dinuclear Zinc(II) Complex and DNA Oligomers as Novel Sequence-Selective Artificial Ribonucleases[**]

Shigeo Matsuda, Akira Ishikubo, Akinori Kuzuya, Morio Yashiro, and Makoto Komiyama[*]

The sequence-selective scission of RNA has been attracting interest because of its potential applications, both in vivo and in vitro.[1] Artificial ribonucleases have been prepared by attaching catalytically active metal ions (mostly lanthanide ions) to DNA oligomers as sequence-recognizing moieties.[2–4] However, the following limitations remain: 1) divalent metal ions such as Zn^{II} and Mg^{II}, which are widely spread in vivo,[5] cannot be used, and 2) selective scission can be achieved only when free metal ions are absent in the reaction mixtures (otherwise, nonselective scission becomes dominant). Further progress is desirable.

It has previously been shown that dinuclear Zn^{II} complexes hydrolyze RNA under physiological conditions.[6, 7] Although the Zn^{II} ion itself is a rather poor candidate for RNA hydrolysis, a notable activity appears when two of them cooperate. Here we report that conjugates of a dinuclear Zn^{II} complex and DNA oligomers selectively hydrolyze RNA at the target site, even in the presence of a considerable amount of free Zn^{II} ions.

Phosphoramidite monomer **1** containing a *N*,*N*,*N'*,*N'*-tetrakis(2-pyridylmethyl)-3,5-bis(aminomethyl)benzene (TPBA) group was prepared according to Scheme 1. By use of **1** and



1

Scheme 1. a) K_2CO_3, [18]crown-6, THF, reflux, 26 h; b) CBr_4, PPh_3, THF, 0 °C, 1 min; c) *N*,*N*-bis(2-pyridylmethyl)amine, *N*,*N*-diisopropylethylamine, CH_3CN, room temperature (RT), 20 h; d) tetrabutylammonium fluoride, THF, RT, 1 h; e) $(iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, CH_3CN, RT, 90 min. TBDPS = *tert*-butyldiphenylsilyl.

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[*] Prof. Dr. M. Komiyama, S. Matsuda, A. Ishikubo, A. Kuzuya,
Prof. Dr. M. Yashiro
Department of Chemistry and Biotechnology
Graduate School of Engineering, The University of Tokyo
Hongo, Tokyo 113-8656 (Japan)
Fax: (+81) 3-5802-3340
E-mail: mkomi@chembio.t.u-tokyo.ac.jp

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the conventional phosphoramidite monomers on an automated synthesizer, TPBA residues were tethered to the 5′ ends of DNA oligomers through a hexamethylene linker. The hydrolysis of RNA (labeled with ^{32}P at the 5′ end) by a mixture of the modified DNA (TPBA-DNA) and Zn^{II} ions was carried out at pH 7.0 and 37 °C, and analyzed by polyacrylamide gel electrophoresis.

Figure 1 shows the typical gel-electrophoresis patterns obtained. The DNA portion in TPBA-DNA is complementary to the U4-C23 part in the RNA substrate (Figure 2). When the feed ratio of Zn^{II} ions to TPBA-DNA is 2, a highly selective scission takes place at the 3′ side of C24 (Figure 1, lane 7; the arrow in Figure 2 shows the site). The band comigrates with the corresponding band for alkaline hydrolysis in lane 2. When the DNA in TPBA-DNA is complementary to the U2-A21 portion in the RNA (in place of the U4-C23 portion), selective scission occurs at the 3′ side of U22. When the DNA is complementary to the A1-G20 part in RNA, the 3′ sides of A21 and U22 are selectively hydrolyzed.

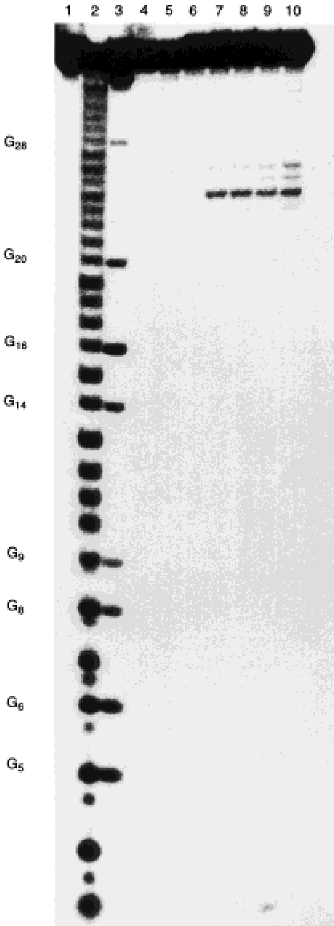

Figure 1. Autoradiographs for the sequence-selective hydrolysis of RNA (labeled with ^{32}P at the 5′ end) by the Zn^{II} complex of TPBA-DNA at pH 7.0 and 37 °C for 3 h: lane 1: no treatment; lane 2: alkaline hydrolysis; lane 3: hydrolysis by RNase T_1 (G-specific); lane 4: treatment with Zn^{II} only (5 μM); lane 5: treatment with a 2:1 mixture of Zn^{II} and TPBA which is not bound to DNA ($[Zn^{II}]_0 = 700$ μM); lanes 6–10: treatment with Zn^{II}/TPBA-DNA mixtures ($[Zn^{II}]_0/[\text{TPBA-DNA}]_0 = 1$, 2, 10, 50, and 100, respectively); $[\text{RNA}]_0 = 0.2$, $[\text{TPBA-DNA}]_0 = 5$ μM. The extent of RNA scission in lane 7 is around 5%.

1 10 20 ↓ 30
5' AUC UGG AGG UCC UGU GUU CGA UCC ACA GAA UUC GUC 3' RNA

3' ACC TCC AGG ACA CAA GCT AG**X** 5' TPBA-DNA

Figure 2. Sequences of the TPBA-DNA and substrate RNA used for the experiment in Figure 1. X is a residue carrying TPBA. The arrow indicates the site of selective scission.

Thus, sequence-selective ribonucleases employing Zn^{II} ions as catalytic centers have been prepared for the first time.

Quite importantly, the sequence selectivity for the scission is satisfactorily high, even when a large amount of free Zn^{II} ions exists in the reaction mixtures (see lanes 8–10 of Figure 1, where $[Zn^{II}]_0/[\text{TPBA-DNA}]_0 = 10$, 50, and 100, respectively; the subscripted 0 indicates initial concentration). The free Zn^{II} ions have no significant effect on the sequence-selective RNA scission.[8] It is also noteworthy that no measurable scission occurs when $[Zn^{II}]_0/[\text{TPBA-DNA}]_0 = 1$ (lane 6). The 2:1 Zn^{II}–TPBA complex in the artificial enzyme is responsible for the selective scission. This argument is further supported by the fact that the DNA conjugates with a *N,N*-bis(2-pyridylmethyl)amine residue, which forms a mononuclear Zn^{II} complex, are virtually inactive at pH 7.0 and 37 °C (see the supporting information).

When a TPBA molecule (not bound to DNA) is used in place of TPBA-DNA, no RNA hydrolysis is observed, even with $[Zn^{II}]_0 = 700$ μM (lane 5; $[\text{TPBA}]_0 = 350$ μM). Thus, selective RNA hydrolysis requires the formation of a heteroduplex, in which the Zn^{II}–TPBA complex is correctly placed near the target phosphodiester linkage (the selective reaction in lane 7 is achieved with $[Zn^{II}]_0 = 5$ μM). The free Zn^{II} ion shows no measurable RNA scission, at least under the conditions employed (lane 4).

The overwhelmingly important role of the 2:1 Zn^{II}–TPBA complex is further evidenced in the hydrolysis of adenylyl-(3′→5′)adenosine (ApA) by the combination of Zn^{II} and TPBA (which is not bound to DNA). There, $[Zn^{II}]_0$ is varied while $[\text{TPBA}]_0$ is kept constant at 2.5 mM. As expected, the rate of hydrolysis sigmoidally increases with increasing $[Zn^{II}]_0$, and attains a plateau at $[Zn^{II}]_0/[\text{TPBA}]_0 = 2$ (see the supporting information). Furthermore, the formation of a 2:1 complex has been confirmed by ^1H NMR spectroscopy.[9] The present artificial ribonucleases involving the dinuclear Zn^{II} complex as catalytic centers are promising for a variety of applications.

Experimental Section

The phosphoramidite monomer **1** and all of the reaction intermediates in Scheme 1 were characterized by ^1H and ^{31}P NMR spectroscopy. The sample of TPBA-DNA used for the experiment presented in Figure 1 was analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry: calcd: 6738.64; found: 6738.8. An HPLC analysis of the enzymatic digestion of TPBA-DNA was in fair agreement with the structure.

The RNA cleavage was carried out in 50 mM Tris-HCl buffer (pH 7.0; Tris = tris(hydroxymethyl)aminomethane) containing 0.2 μM RNA, 5 μM TPBA-DNA, 100 mM NaCl, and the required amount of $Zn(NO_3)_2$. The reaction mixture was heated to 85 °C for 1 min, and cooled to the reaction temperature (37 °C). After the reaction, the mixture was loaded onto a

15% denaturing polyacrylamide gel. The ApA hydrolysis by the 2:1 Zn$^{\rm II}$–TPBA complex was followed by reverse-phase HPLC. The 2′,3′-cyclic monophosphate of adenosine as the intermediate was rapidly hydrolyzed to the 2′- and 3′-monophosphates; thus, not much intermediate accumulated.

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[8] The weak bands in the 3′ side of the main scission site in lane 10 are tentatively ascribed to catalysis by zinc(II) hydroxide clusters formed at a high Zn$^{\rm II}$ concentration ($[{\rm Zn^{II}}]_0 = 0.5\,{\rm mM}$).

[9] When $[{\rm Zn^{II}}]_0 = [{\rm TPBA}]_0 = 2.5\,{\rm mM}$ in D_2O, TPBA gave one set of ^{1}H NMR signals which differed from those of the free ligand. Upon increasing the $[{\rm Zn^{II}}]_0/[{\rm TPBA}]_0$ ratio, another set of signals (corresponding to the 2:1 complex) gradually appeared. At a ratio of 2, only signals for the 2:1 complex were observed.

An STM Study of Chemically Deposited Silver Nanoclusters on Mixed Self-Assembled Monolayers[**]

Ulrich-Walter Grummt,* Matthias Geissler, Till Drechsler, Harald Fuchs, and Ralf Staub

Single electron tunneling (SET) processes in quantum-confined low-dimensional systems have gained increasing interest over recent years.[1, 2] Understanding of the mechanism of charge transport through single molecules is a prerequisite for the rational design of electronic devices based on single molecules.[3] A challenging problem is to establish metallic contacts in a stable and reproducible way to individual molecules. Mixed self-assembled monolayers (SAMs) containing a small number of the molecules to be studied as guests in a matrix of inert host molecules offer the possibility to address single molecules by the STM tip (STM = scanning tunneling microscopy). The basic idea of the present study is to generate silver clusters as small as Ag_4 in the immediate vicinity of a four-electron reducing agent which is incorporated in a monolayer of long-chain alkanethiols (Figure 1).[4] In connection with an STM tip the silver nanoclusters provide a vertical double tunnel junction, which should allow the observation of SET processes, even at room temperature if the cluster capacitance is sufficiently small ($e^2/2C \gg kT$). Chemical deposition of silver clusters on SAMs is a simple and more gentle alternative to deposition from a cluster beam[5] and evaporation techniques.[6]

A series of aromatic methyl sulfides were synthesized bearing two adjacent terminal hydroquinone units. For this study the compound BHQ1 (Figure 1) was selected to form mixed SAMs with an excess of *n*-decanethiol (nDT). On Au(111) nDT forms layers with a hexagonal $(\sqrt{3}\times\sqrt{3})\mathrm{R}30^\circ$ structure.[7] Molecular resolution was achieved with pure nDT SAMs. All variants of superlattices described by Delamarche et al. could be observed.[8] The MM3 simulations of SAMs where one of 18 nDT molecules was replaced with BHQ1 predict that BHQ1 should protrude beyond the nDT surface. However, not unexpectedly, single molecules of BHQ1 could not be resolved. On the other hand, phase segregation of nDT and BHQ1 to form separate domains could be ruled out.

The treatment of the mixed SAMs with an aqueous ammonia-containing solution of silver nitrate (5 M $AgNO_3$ in water/ethanol 2/1, pH 10) results in the formation of silver

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[*] Prof. Dr. U.-W. Grummt, Dipl.-Chem. M. Geissler
Institut für Physikalische Chemie der Universität
Lessingstrasse 10, D-07743 Jena (Germany)
Fax: (+49) 3641-948302
E-mail: cug@pc04.chemie.uni-jena.de

Dipl.-Phys. T. Drechsler, Prof. Dr. H. Fuchs
Physikalisches Institut der Universität Münster (Germany)

R. Staub
Institut für Angewandte Photophysik der Technischen Universität Dresden (Germany)

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