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Phosphodiester bond cleavage mediated by a cyclic β-sheet peptide-based dinuclear zinc(II) complex

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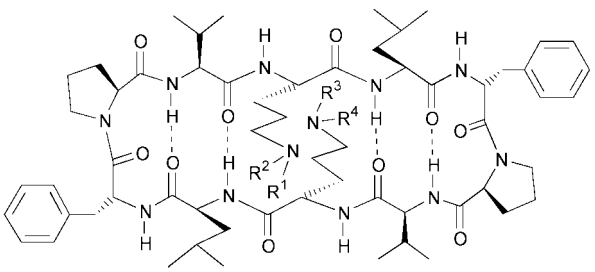
A dinuclear Zn(II) complex of $N^{2\delta}$,$N^{2\delta}$,$N^{2'\delta}$,$N^{2'\delta}$-tetrakis(2-pyridylmethyl) derivative of cyclic peptide gramicidin S markedly accelerated the cleavage of the phosphodiester linkage of the RNA model substrate 2-hydroxypropyl *p*-nitrophenyl phosphate.

Rational design of functional molecules mimicking biologically important species such as enzymes is one of the most challenging fields in modern chemistry. Peptides and peptidomimetics are promising building blocks for the construction of such systems. While numerous studies using α-helical peptides as well-defined structural units were reported, functional molecules based on β-structured peptides were few. The cyclic decapeptide gramicidin S (GS, Fig. 1) possesses a stable antiparallel β-sheet conformation with two type II′ β-turns at the D-Phe-Pro sequences.[1] The amino groups of the two Orn residues of GS are located at one side of the β-sheet, and are suitable for the introduction of various functional groups.

It has been shown that the active sites of many enzymes contain two metal ions which operate cooperatively.[2] Artificial models for the dinuclear metalloenzymes were reported in which pyridine-containing ligand groups were linked to a molecular scaffold such as a calix[4]arene framework.[3] Since the dinuclear Zn(II) complex of the simple diamine derivative $[(PyCH_2)_2NCH_2]_2CHOH$ was shown to promote hydrolysis of the phosphodiester linkage of a diribonucleotide,[4] we have prepared a GS derivative containing two bis(2-pyridylmethyl) amino groups in place of free amino groups, namely [Orn$(PyCH_2)_2^{2,2'}$]GS (**1**). In the present study, the Zn(II) ion-chelating behavior of **1** and phosphodiester bond cleavage mediated by the dinuclear Zn(II) complex have been examined.

$N^{2\delta}$,$N^{2\delta}$,$N^{2'\delta}$,$N^{2'\delta}$-Tetrakis($PyCH_2$) derivative **1** was prepared from GS•2HCl in 57% yield by treatment with 2-PyCHO and $NaBH_3CN$ in MeOH.[5] For comparison, a GS derivative containing only one $(PyCH_2)_2N$ group, namely [Orn$(PyCH_2)_2^2$, Orn(Tfa)$^{2'}$]GS (**2**), was prepared in a similar manner from the singly protected [Orn(Tfa)^2]GS.[6] An alternative bis($PyCH_2$) derivative in which each Orn side chain carries a $PyCH_2NH$ group, namely [Orn$(PyCH_2)^{2,2'}$]GS (**3**), was also prepared by treatment of GS•2HCl with PyCHO followed by catalytic hydrogenation in 70% yield.



GS	$R^1 = R^2 = R^3 = R^4 = H$
1	$R^1 = R^2 = R^3 = R^4 = PyCH_2$
2	$R^1 = R^2 = PyCH_2$, R^3 = Tfa, $R^4 = H$
3	$R^1 = R^3 = PyCH_2$, $R^2 = R^4 = H$

Fig. 1 The structures of gramicidin S (GS) and the 2-pyridylmethyl ($PyCH_2$) derivatives **1**–**3**.

The pyridylmethyl derivatives **1**–**3** exhibited similar CD spectra to that of the parent GS, although the molecular ellipticity of **1** was slightly smaller than GS (Fig. 2a). The ^1H NMR spectral characteristics of **1** in DMSO-d_6 were also similar to those of GS, but the NH proton signal of the D-Phe residue of **1** (δ 8.59, d, J = 4.8) was more shielded, possessing a larger $N^{\alpha}H$–$C^{\alpha}H$ J value compared with the corresponding signal for GS (δ 9.04, d, J = 2.8). A similar spectral feature was observed for the GS derivatives lacking a main chain-side chain H-bonding interaction between $N^{\delta}H$(Orn) and C=O (D-Phe).[7,8] Therefore **1** was assumed to adopt essentially the same β-sheet conformation as that of GS, although the conformation was slightly distorted or disordered due to the absence of the H-bonding stabilization.

Zn(II) ion-binding properties of these pyridylmethyl derivatives were studied by spectrometric titrations. A CD spectral change between 245 and 280 nm upon the addition of aliquots of $ZnCl_2$ to the solution of **1** in MeOH as shown in Fig. 2b suggested stepwise formation of the 1:1 and 1:2 complexes between **1** and Zn^{2+}. A similar spectral change was also observed in buffered aqueous CH_3CN (pH 7.0) which was employed as the solvent for kinetic experiments described later. Formation of the stable dinuclear complex **1**•$(Zn^{2+})_2$ was further supported by UV spectroscopic titration. The CD spectrum of the dinuclear complex‡ in MeOH below 250 nm which was assumed to reflect the main chain conformation was similar to the CD spectral curve of unmodified GS (Fig. 2a). ^1H NMR analysis in DMSO-d_6 indicated a C_2 symmetrical structure for the dinuclear Zn(II) complex **1**•$(Zn^{2+})_2$. ROESY experiments revealed steric proximity of one of the pyridyl groups in each side chain of the Orn residues and the α-protons in the Pro-Val sequences. The NH proton of the D-Phe residue exhibited a similar signal (δ 9.06, d, J < 3.0) to that of natural GS. Taking into account these spectral characteristics the dinuclear complex was assumed to adopt the stable GS-type β-sheet conformation. Instead of the *i* to *i* + 2 type

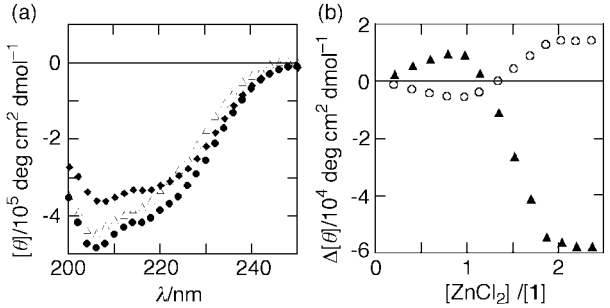


Fig. 2 (a) CD spectra in MeOH of GS•2HCl (●), **1** (◆) and **1** with Zn^{2+}: $[ZnCl_2]/[\mathbf{1}]$ = 2.4 (△). (b) Change of [θ] at 250 nm (○) and 271 nm (▲) upon the addition of $ZnCl_2$ to **1** in MeOH.

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Table 1 Pseudo-first-order rate constant of Zn(II) complex-mediated phosphodiester bond cleavage of HPNP[a]

Complex	k_{obs}/s^{-1}	Relative rate
None	0.51	1
1•$(Zn^{2+})_2$	3300	6500
2•Zn^{2+}	11	22
4•$(Zn^{2+})_2$	41	80

[a] Assays were carried out in 50% CH_3CN–20 mM HEPES buffer (pH 7.0) except **2**•Zn^{2+} (80% CH_3CN–20 mM HEPES buffer).

$N^{\delta}H$(Orn)··· C=O(D-Phe) H-bonding found in GS and its derivatives[8] the metal ions might play some role in the conformational stabilization. As expected, the bis($PyCH_2$) derivative **2** formed only the mononuclear 1 : 1 complex and no complex-forming tendency with Zn^{2+} was observed for the other derivative **3**.

Activity of the Zn(II) complexes of **1** and **2** for the cleavage of the linkage of 2-hydroxypropyl *p*-nitrophenyl hydrogen phosphate (HPNP) as an RNA model substrate was examined. For comparison, a dinuclear Zn(II) complex of the propanediamine derivative $[(PyCH_2)_2NCH_2]_2CH_2$ **4** lacking the peptide moiety was prepared and was also subjected to the kinetic study. The complexes were generated *in situ* in buffered aq. CH_3CN solution (pH 7.0) and the reaction progress was monitored by the absorbance at 400 nm due to the liberated *p*-nitrophenolate ion from HPNP.§ The obtained pseudo-first-order rate constants (k_{obs}) of the Zn(II) complex-mediated reaction are summarized in Table 1. The dinuclear complex **1**•$(Zn^{2+})_2$ was found to accelerate the reaction drastically. The effect of the mononuclear complex **2**•Zn^{2+} was much lower, indicating the importance of the cooperative participation of the two Zn(II) centers in the reaction process. Since the rate constant of the dinuclear complex **4**•$(Zn^{2+})_2$ lacking the peptide moiety was the same order as that of the mononuclear complex, the relative arrangement of the two Zn(II) centers linked to the rigid β-sheet framework by a moderately flexible trimethylene chain was considered to furnish a desirable reaction site enabling cooperative functioning of the two metal ions.

The pH dependence study of the reaction rate using **1**•$(Zn^{2+})_2$ demonstrated the optimal activity at pH 7.0. Metal ion-bound water in a hydrophobic environment is known to be highly acidic ($pK_a \approx 7$ to 8)[9] and in our case the Zn^{2+}–OH^- species generated near the antiparallel β-strands of GS moiety acts as a nucleophile, attacking the β-OH group of HPNP. As shown in Fig. 3 another Zn(II) ion was assumed to coordinate to phosphate oxygen, assisting the nucleophilic attack of the resulting anionic β-oxygen atom in HPNP to accomplish the intramolecular transesterification. The presence of the neighbouring hydroxy group was essential since the complex did not enhance the hydrolysis of di(*p*-nitrophenyl) hydrogen phosphate examined as a DNA model substrate.

In summary, we have demonstrated that the dinuclear Zn(II) complex **1**•$(Zn^{2+})_2$ possessing a β-sheet framework remarkably promoted phosphodiester bond cleavage of the RNA model substrate HPNP.¶ Very recently, Scrimin *et al.* reported transesterification of HPNP by a dinuclear Zn(II) catalyst based on water-soluble 3[10]-helical peptide.[10] A cyclodextrin-based dinuclear Cu(II) complex which hydrolyzes an amide bond was reported by Fujita's group.[11]

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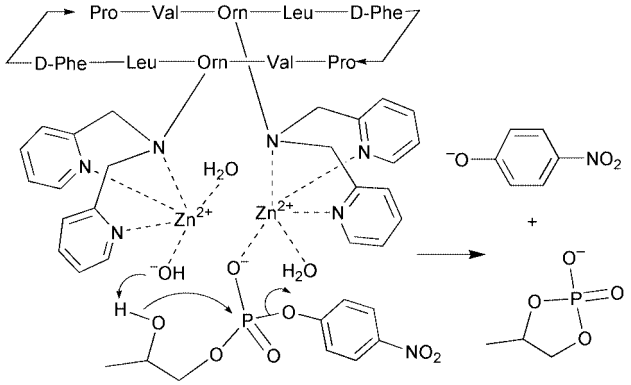

Fig. 3 A possible mechanism for the transesterification of HPNP mediated by the dinuclear Zn(II) complex of **1**.

Notes and references

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‡ **1**•$(Zn^{2+})_2$ was prepared from a MeOH solution of **1** (26 mg, 0.017 mmol) and $Zn(NO_3)_2$•$6H_2O$ (17 mM, 2 ml) by evaporating the solvent and lyophilization as a white solid (33 mg). ESI-MS: *m/z* 817.4 [**1** + 2Zn − 2H]$^{2+}$, $(C_{84}H_{111}N_{16}O_{10}Zn_2)^{2+}$ requires *m/z* 817.4. The complex thus obtained was water-soluble although **1** itself was almost insoluble in water.

§ A typical kinetic experiment was undertaken in 50% CH_3CN–20 mM HEPES buffer (pH 7.0) as follows: After mixing the solutions of **1** (0.5 mM, 3.0 ml) and $Zn(NO_3)_2$ (50 mM, 60 μl) for 1 min, HPNP (75 mM, 10 μl) was added and the pseudo-first-order rate constant k_{obs} was calculated by initial slope method using the absorbance at 400 nm.

¶ The corresponding D-Tyr analog of **1**, namely [Orn$(PyCH_2)_2^{2,2'}$, D-Tyr$^{4,4'}$]GS, possessing phenolic hydroxy groups to which a variety of functional groups (*e.g.* substrate binding sites) could be introduced was also prepared. The dinuclear Zn(II) complex exhibited similar activity to **1**•$(Zn^{2+})_2$ (k_{obs} = 2200 s^{-1}).

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