Photo-Regulation of RNA Hydrolysis by the Zinc(II) Complex Carrying Azobenzene

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The first photo-regulation of non-enzymatic RNA hydrolysis has been achieved. A positively-charged and bulky regulator has been linked to a catalytically active Zn(II) complex, via a photo-responsive linker involving an azobenzene. The rate of RNA hydrolysis has been reversibly modulated by photo-irradiation.

Non-enzymatic hydrolysis of RNA is potent for a variety of applications, and a number of active catalysts have been already reported.[1] However, little has been known yet on the control of catalytic activity. If one can make the catalysis occur at a predetermined place and timing, it should greatly widen the scope of application either *in vitro* or *in vivo*.

The strategy we propose here is to tether a catalytic metal complex to a positively-charged and bulky periphery ("regulator"), via a linker which alters the structure in response to an outer stimulus. As long as the regulator is located at some distance from the catalytic center, it accelerates RNA hydrolysis as an electrostatic catalyst (see Figure 1 (A)).[2] When the regulator is placed too close to the catalytic center, however, the reaction is suppressed by steric hindrance (B). Thus, the catalytic activity can be modulated by using the stimulus. This paper reports on photo-regulation of the catalytic activity of the Zn(II) complex of ligand **1**, which involves photo-responsive azobenzene in the linker.[3-5]

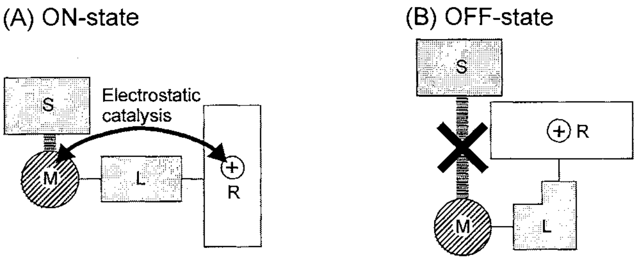
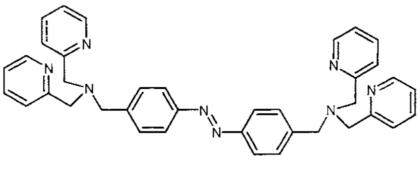


Figure 1. The strategy for the regulation of RNA hydrolysis by outer stimulus; M = metal center, R = regulator, S = substrate. The linker L alters the structure in response to the stimulus.

Ligand **1** was synthesized by the reaction of 4,4'-bis(bromomethyl)azobenzene[6] with bis[(2-pyridyl)methyl] amine (BPA). The product was purified by silica-gel column chromatography, and characterized by ^{1}H-NMR spectroscopy. Required amounts of **1** (in ethanol) and $Zn(NO_3)_2$ were added to 50 mM Hepes buffer (the reaction mixtures contained 1 vol.% ethanol). The hydrolysis of adenylyl(3'-5')adenosine (ApA) (the initial concentration = 0.1 mM) was monitored by reversed-phase HPLC (Merck LiChrosphere RP-18(e) ODS column; water/acetonitrile = 92/8). Photo-isomerization of the azobenzene was achieved by irradiating the light from a 150 W xenon lamp through an appropriate color-glass filter. Infrared light was cut off by water filter.



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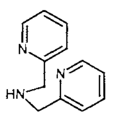

BPA

As shown in Figure 2, the 2:1 mixture of Zn(II) and **1** effectively hydrolyzed ApA to adenosine and its monophosphates (the term I).[7] The pseudo-first-order rate constant was 0.35 h^{-1}, under the conditions that $[Zn(II)]_0 = 2$ and $[1]_0 = 1$ mM at pH 7.2 and 50 °C. It is noteworthy that this mixture is far more (> 70 fold) active than is the 1:1 complex between Zn(II) and BPA.[8] The 2:1 Zn(II)/**1** complex, which is almost quantitatively formed in the solution,[9] has a significant activity for RNA hydrolysis.

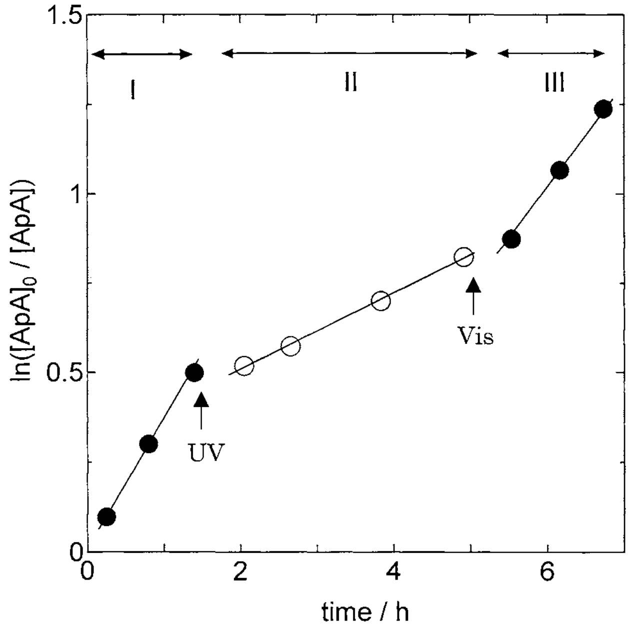

Figure 2. Photo-regulation of the ApA hydrolysis by the 2:1 Zn(II)/**1** mixture at pH 7.2 and 50 °C; $[Zn(II)]_0 = 2$ and $[1]_0 = 1$ mM. At the point designated by the arrow, either UV light (300 nm < λ < 400 nm) or visible light (λ > 420 nm) was irradiated for 30 min.

When the solution was irradiated with UV light (300 nm <

$\lambda < 400$ nm) for 30 min, the hydrolysis rate notably decreased (the term II in Figure 2). The rate constant was 0.11 h^{-1}. In the absorption spectra, the band around 350 nm (assignable to the trans-form of the azobenzene) was diminished, and a new band (for the cis-form) appeared around 450 nm. Thus, the trans→cis isomerization of the azobenzene is responsible for the UV-induced deceleration of the hydrolysis. When the mixture was further irradiated with visible light ($\lambda > 420$ nm), the catalytic activity was restored and the rate constant was increased to 0.31 h^{-1} (the term III). Concurrently, the absorption band at 450 nm weakened and the band at 350 nm increased. The cycle involving the activation of RNA hydrolysis by visible light and the deactivation by UV light was repeated many times without apparent deterioration.

The fractions of the cis- and the trans-isomers of the 2:1 Zn(II)/**1** complex in the reaction mixtures were evaluated by ^{1}H-NMR.[10] The virgin sample (before the photo-irradiation) was an almost pure trans-isomer. After irradiating UV light and visible light for 30 min, however, the cis:trans ratios were 8:2 and 2:8, respectively. By using these ratios, the results in Figure 2 have been analyzed. It has been concluded that only the trans-isomer of the complex is active for the RNA hydrolysis and the cis-isomer is virtually inactive. The slow ApA hydrolysis in the term II (after the UV irradiation) is mostly ascribed to the catalysis by the trans-isomer, which exists as the minor component in the solution. When the azobenzene in the complex takes the trans-form, the two Zn(II) ions are located far away from each other and behave independently. One of them functions as the catalytic center for ApA hydrolysis, and the other promotes the reaction as electrostatic catalyst. On the isomerization of the azobenzene to cis-structure, the two Zn(II) ions come closer. Here, the coordination of the substrate ApA to one of the two Zn(II) ions (the catalytic center) is sterically hindered by the bulky moieties coordinating to the other Zn(II) ion, and thus the catalytic activity is almost nil. It is unlikely that the two Zn(II) ions in the trans-isomer show the acid/base cooperative catalysis. According to molecular-modeling study, the distance between these two Zn(II) ions is greater than 10 Å, and too large for the cooperation.[11]

In conclusion, the rate of RNA hydrolysis has been reversibly photo-controlled by using the Zn(II) complexes involving an azobenzene residue in the ligand.

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7 The 3'-monophosphate was predominantly (>90%) formed over the 2'-monophosphate. The 2',3'-cyclic monophosphate was rapidly hydrolyzed and not much accumulated.

8 The rate constant for the ApA hydrolysis by the 1:1 Zn(II)/BPA complex (2 mM) is smaller than 0.005 h^{-1}.

9 More than 99% of the Zn(II) ions in the reaction mixture form complexes with either of the two BPA residues of 1, as estimated from the binding constant of the 1:1 Zn(II)/BPA complex; "Stability Constants of Metal-Ion Complexes, Supplement No.1 Special Publication 25," ed by A. E. Martell, The Chemical Society, Burlington House, London (1971), part II, p. 678.

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