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Metal ion induced allosteric transition in the catalytic activity of an artificial phosphodiesterase†

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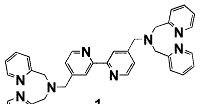
An artificial phosphodiesterase (1) bearing two kinds of metal binding sites, a catalytic site and a regulatory bipyridine site showed a unique allosteric transition in the catalytic activity against the metal concentration.

The design of artificial allosteric systems is of great significance for regulating the catalytic activities and complexation properties of artificial receptors.[1] We have been interested in the exploitation of homotropic allosteric systems, which show the nonlinear amplification of binding events and chemical signals.[2] This phenomenon is useful to control the activities of an artificial receptor in an OFF–ON switching manner at a threshold condition.[1,2] Allosteric molecular recognition systems have been studied well, whereas studies on artificial allosteric catalytic systems are still very rare.[3,4] Furthermore, to our best knowledge, there is no report that the same metal ion plays both roles of a catalytic metal ion and an effector metal ion, giving rise to an allosteric transition phenomenon.[4,5] Such a novel system would show a unique catalytic activity response depending on the metal ion concentration.

There is considerable interest in creating artificial phosphodiesterases because of their potential application in gene therapy. For this purpose, numerous catalysts containing essential metal ions have been explored.[6] Here we designed compound **1** as an allosteric artificial phosphodiesterase, which has two different types of metal ion binding sites, *viz.* a 2,2′-bipyridine (Bpy) as a regulatory site and 2,2′-dipicolylamine (DPA) as catalytic sites.[7] The DPA moieties have higher Zn^{2+} or Cu^{2+} affinities than the Bpy moiety.[8] Hence one can predict that the first two metal ions are bound to two DPA moieties in **1** to produce $\mathbf{1}\cdot(M^{2+})_2$ (M^{2+}: Zn^{2+} or Cu^{2+}). The regulatory site, the Bpy moiety, adopts mostly a transoid conformation because of the repulsion between lone-pairs of nitrogen atoms,[9] where the amine distance between DPA is estimated to be 0.84 nm (catalytically less active). The metal ion coordination to Bpy can induce a conformational change from transoid to cisoid enforcing an alignment of the DPA sites at a distance of 0.48 nm; that is '*an allosteric transition*' (Fig. 1). Reference compounds **2** and **3** should not show such an allosteric transition phenomenon.

The complexation behaviour of **1** (0.4 mM) with Zn^{2+} (as a perchlorate salt) in ethanol/water (HEPES, 16 mM) = 1:2 v/v solution at pH 7.7 and 25 °C was monitored by photometric titration. Binding processes of Zn^{2+} to **1** were accompanied by three steps of spectral changes with isosbestic points observed at 293.2 nm (0 to 1 equiv. Zn^{2+}), 296.8 nm (1 to 2 equiv. Zn^{2+}) and 297.4 nm (more than 2 equiv. Zn^{2+}). The absorbance of DPA moieties changed almost linearly upon addition of 0 to 1 equiv. Zn^{2+} and 1 to 2 equiv. Zn^{2+}. This result clearly shows that the first two Zn^{2+} ions are bound to the DPA moieties quantitatively to produce $\mathbf{1}\cdot(Zn^{2+})_2$ under these conditions. At $[Zn^{2+}]$ higher than 2 equiv., a typical shift of the absorption band of the Bpy moiety from 284.6 nm to 312.0 nm was observed, indicating that $Bpy\cdot Zn^{2+}$ is formed at this stage. A plot of the absorbance at 312.0 nm against Zn^{2+} concentration showed a saturation behaviour and the association constant for the formation of $\mathbf{1}\cdot(Zn^{2+})_3$ from $\mathbf{1}\cdot(Zn^{2+})_2$ was evaluated to be 9.1×10^2 M^{-1}. Similar results were obtained for the **1** (1.0 mM) and Cu^{2+} system, where 2 equiv. of Cu^{2+} are bound to the DPA moieties in **1** quantitatively. The association constants for the formation of $\mathbf{1}\cdot(Cu^{2+})_3$ and $\mathbf{1}_2\cdot(Cu^{2+})_5$ from $\mathbf{1}\cdot(Cu^{2+})_2$ were evaluated to be 6.3×10^4 M^{-1} and 1.0×10^8 M^{-2}, respectively. These complexation process were confirmed by ESI-MS and ^{1}H-NMR spectroscopies (see supplementary information†).



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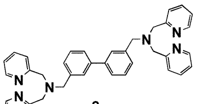

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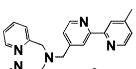

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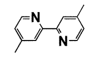

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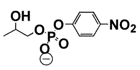

HPNP

We employed 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP) as a substrate.[10] Kinetic experiments were conducted on the basis of the release rate of *p*-nitrophenol from HPNP by monitoring the increase in the absorbance at 406 nm. At first, the kinetic studies at varying Zn^{2+} and Cu^{2+} concentrations were performed and pseudo-first-order rate constants (k_{obs}) were evaluated for **1** and **2** (Figs. 2 and 3). Very interestingly, in the case of **1**, there was a further significant increase in the catalytic activity upon addition of more than 2 equiv. metal ion, whereas such enhancement was not observed for **2** without the regulatory site

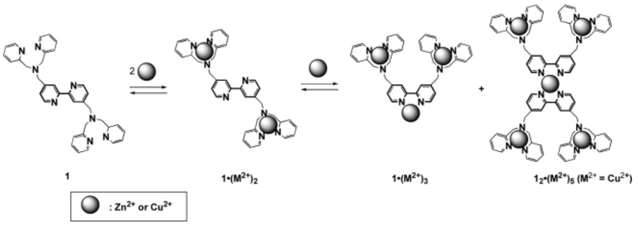

Fig. 1 Schematic representation of allosteric transition of **1**.

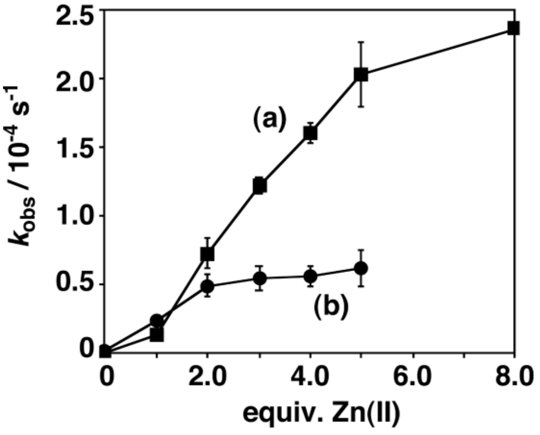

Fig. 2 Plots of pseudo-first-order rate constants (k_{obs}) for the hydrolysis of HPNP (0.8 mM) at various Zn^{2+} concentrations in 33% ethanol/water (HEPES, 25 mM): (a) [**1**] = 0.4 mM, (b) [**2**] = 0.4 mM, pH 7.7 at 25 °C.

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† Electronic supplementary information (ESI) available: synthesis of **1**, characterization of complexes by ^{1}H-NMR and ESI-MS spectroscopies and the analysis of the kinetic data. See http://www.rsc.org/suppdata/cc/b3/b314032f/

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(Figs. 2 and 3). In Fig. 2, the rate constant for **1** with 8 equiv. Zn^{2+} is 3.3 times larger than that for **1** with 2 equiv. Zn^{2+} (**1**$\cdot(Zn^{2+})_2$). The saturation behaviour observed for **1** in Fig. 2 is complementary to the results of photometric titration. In the case of **1** and Cu^{2+}, 19.4 times larger k_{obs} value was evaluated for **1** with 3 equiv. Cu^{2+} against **1** with 2 equiv. Cu^{2+} (**1**$\cdot(Cu^{2+})_2$) (Fig. 3). For **1** with 3 equiv. Cu^{2+}, the ratio of [**1**$\cdot(Cu^{2+})_3$]/[**1**$_2\cdot(Cu^{2+})_5$]/[**1**$\cdot(Cu^{2+})_2$] is estimated to be 81/11/8 by the association constants between Cu^{2+} and the Bpy moiety in **1**.[11] These results indicate that **1**$\cdot(M^{2+})_3$ and/or **1**$_2\cdot(M^{2+})_5$ have higher catalytic activity than **1**$\cdot(M^{2+})_2$.

In order to evaluate the contribution of the Bpy$\cdot Zn^{2+}$ complex to the hydrolysis of HPNP, k_{obs} values with control compounds **3** and **4** were measured. It was found that k_{obs} value of 6.6×10^{-5} s^{-1}obtained from a mixture of **2**$\cdot(Zn^{2+})_2$ and **4**$\cdot Zn^{2+}$ is almost same as that obtained form **2**$\cdot(Zn^{2+})_2$. In addition, the k_{obs} value of 3.5×10^{-5} s^{-1} for **3**$\cdot(Zn^{2+})_2$ is 5.8 times smaller than that for **1**$\cdot(Zn^{2+})_3$. These results show that inter- or intra-molecular reaction catalysed by Bpy$\cdot Zn^{2+}$ and Dpa$\cdot Zn^{2+}$ complexes is not so effective for the hydrolysis of HPNP. Similar results were obtained from Cu^{2+} (see supplementary information†). The increase in the catalytic activity is ascribed, therefore, to the allosteric conformational transition of **1** induced by the coordination of effector metal ion to the Bpy moiety as shown in Fig. 1.

Michaelis–Menten kinetic parameters were evaluated from saturation kinetic experiments to obtain a further insight into the rate enhancement observed with **1**. Saturation kinetic curves were obtained from Zn^{2+} and Cu^{2+} catalysts: (a); [**1**] = 0.4 mM, [Zn^{2+}] = 0.8 mM, (b); [**1**] = 0.4 mM, [Zn^{2+}] = 2.0 mM, (c); [**1**] = 1.0 mM, [Cu^{2+}] = 2.0 mM, (d); [**1**] = 1.0 mM, [Cu^{2+}] = 3.0 mM. A Lineweaver–Burk plot was, then, applied to calculate the Michaelis–Menten constant (K_m) and the catalytic constant (k_{cat}) (see supplementary information†). The results are summarized in Table 1. A significant increase in the value of k_{cat} (4.1 times for Zn^{2+} and 55 times for Cu^{2+}) was obtained from the conditions (b) and (d) compared with (a) and (c). These results indicate that the conformational change of **1** induced by the third M^{2+} complexation, that is, allosteric transition, enhances the rate of hydrolysis but not by a change in the substrate affinity. This should be a consequence of the preferable preorganisation of two DPA$\cdot M^{2+}$ complex units toward the hydrolysis, which would facilitate the attack of intramolecular hydroxyl ion sitting on M^{2+} to the substrate.[5,6]

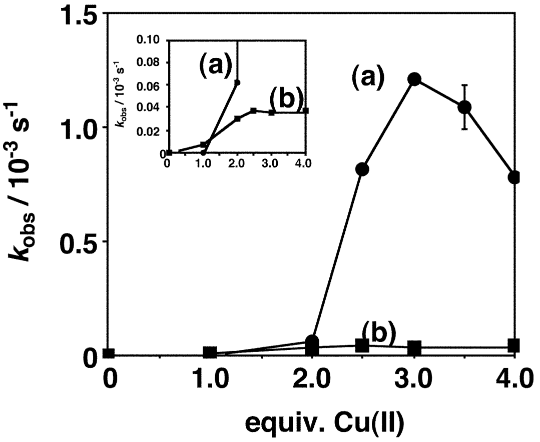

Fig. 3 Plots of pseudo-first-order rate constants (k_{obs}) for the hydrolysis of HPNP (1.0 mM) at various Cu^{2+} concentrations in 33% ethanol/water (HEPES, 25 mM): (a) [**1**] = 1.0 mM, (b) [**2**] = 1.0 mM, pH 7.7 at 25 °C. Inset is an enlarged view for **2** and Cu^{2+}.

Table 1 Michaelis–Menten kinetic parameters

	$10^2\cdot k_{cat}/s^{-1}$	$10^2\cdot K_m$/M
(a) **1** with 2 equiv. Zn^{2+}	0.051	0.19
(b) **1** with 5 equiv. Zn^{2+}	0.21	0.31
(c) **1** with 2 equiv. Cu^{2+}	0.026	0.36
(d) **1** with 3 equiv. Cu^{2+}	1.43	1.27

In conclusion, we have demonstrated that compound **1** bearing two types of Zn^{2+} or Cu^{2+} binding sites shows a novel allosteric response in the catalytic activities toward the hydrolysis of phosphodiester. We anticipate, therefore, that such a supramolecular catalytic system would further produce intelligent artificial systems responding to various kinds of chemical stimuli.

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