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On/off regulation of catalysis by allosteric control of metal complex nuclearity†

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The nature of the allosteric metal ion M (Pd^{2+} or Pt^{2+}) in complexes ML of a polytopic ligand controls uptake of additional Cu^{2+} ions; while $[Cu_2Pd(L\text{-}4H)]^{2+}$ is a highly active catalyst for phosphodiester cleavage, [CuPt(L-4H)] is inactive.

Allosteric[1] modulation of activity is a common feature of biological receptors and enzymes. The use of allosteric interactions enables chemists to control molecular function, an important aspect in the development of functional supramolecular devices of increasing complexity. Only recently the first abiotic allosteric catalysts have been described.[2,3] In one such catalyst[2a–2c] the allosteric metal ion controls the conformation and reactivity of a dinuclear, catalytic site.[2] Depending on the nature of the allosteric metal ion, the cleavage rate of a phosphate ester varies by a factor of up to 70.

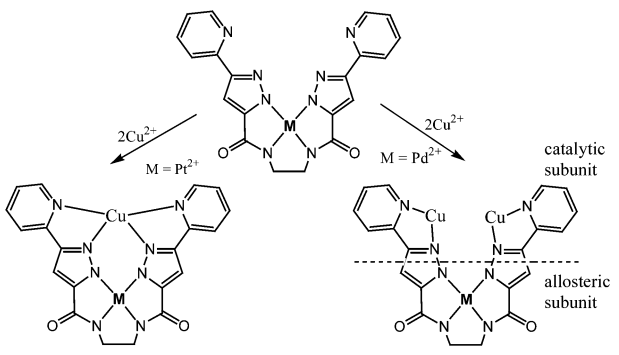
We have now observed in a related polynuclear complex a different type of activity control: the allosteric metal ion controls the uptake of metal ion cofactors which are essential for catalysis. Ligand **L**[4] has an allosteric subunit that coordinates Pd^{2+} or Pt^{2+}, and a catalytic subunit for the binding of either one or two copper(II) ions (Scheme 1). The nuclearity of the complex, *i.e.* formation of either a monocopper(II) or dicopper(II) complex, is dependent on the nature of the allosteric metal ion, and only the dicopper complex is an active catalyst for the cleavage of a phosphodiester.

Allosteric control of the binding of catalytic metal ion cofactors has been reported for enzymatic systems *e.g.* in the case of ribonucleotide reductase which consists of two protein subunits with a diiron active site.[5]

Complexes $Na_2[(Pd(L\text{-}4H)]\cdot 3H_2O$ and $K_2[(Pt(L\text{-}4H)]\cdot 5H_2O$ have been prepared from **L** and metal salts in the presence of base. Spectroscopic and microanalytical data support a structure with tetradentate Pd and Pt coordination by two deprotonated amide and two deprotonated pyrazole nitrogen atoms in the complex dianion. It is shown by electrospray MS that these complexes are inert against metal exchange in the presence of even large excess Cu^{2+}.

We have followed the coordination of Cu^{2+} to $[(Pd(L\text{-}4H)]^{2-}$ (**1**) and $[(Pt(L\text{-}4H)]^{2-}$ (**2**) by spectrophotometric titration of the complexes with copper(II) acetate in 3 : 1 DMSO–H_2O media at pH 7. Surprisingly, the absorbance diagrams indicate that **1** incorporates two, but **2** only one, copper ion (Fig. 1). This behaviour is confirmed by ESI mass spectra of $[M(L\text{-}4H)]^{2-}$–$Cu(OAc)_2$ (M = Pd, Pt) 1:2 mixtures in 3 : 1 DMSO–H_2O media. In particular, in the spectrum of the Pd complex signals of only dicopper complexes $[Cu_2Pd(L\text{-}4H)(OH)]$ (*m*/*z* 649.0) and $[Cu_2Pd(L\text{-}4H)(OH)(OAc)]$ (*m*/*z* 709.1) are observed, while in the spectrum of the Pt complex a signal of only a monocopper complex $[CuPt(L\text{-}4H)]+H^+$ (**4**) is observed (*m*/*z* 657.3). In the EPR spectra of **1**–$Cu(OAc)_2$, both 1 : 1 and 1 : 2 mixtures in DMSO–H_2O glass at 77 K, a rather broad and featureless signal with g = 2.09 G is found. This indicates the formation of the dinuclear complex with intramolecular coupling interaction of the two Cu. In contrast, the EPR spectrum of **2**–$Cu(OAc)_2$ 1 : 1 reveals well resolved peaks ($g_\perp$ = 2.06, $g_\parallel$ = 2.25, $A_\parallel$ = 163 cm^{-1}), typical for mononuclear Cu^{2+} complexes with in-plane coordination by four nitrogen donor atoms.[6] We suggest a tetradentate in-plane coordination of Cu^{2+} by two pyrazole N and two pyridyl N, according to molecular models with significant elongation of the Cu–N_{py} bonds and widening of the N_{py}–Cu–N_{py} angle. On addition of a second equivalent $Cu(OAc)_2$, EPR signals of the 1 : 1 complex overlap with those of free Cu^{2+}, supporting the idea that this complex does not incorporate a second Cu ion. Interestingly, a very similar behaviour is found for a **1**–$CuCl_2$ mixture ($g_\perp$ = 2.06, $g_\parallel$ = 2.25, $A_\parallel$ = 150 cm^{-1}), indicating that acetate stabilizes the dicopper complex and is essential to its formation.

The formation of a dicopper complex $[Cu_2Pd(L\text{-}4H)(\mu\text{-}OH)(\mu\text{-}OAc)]$ (**3**) has been confirmed by X-ray crystallography.[7] The complex (Fig. 2) is nearly planar with exception of the bridging OH coligand that lies 1.14 Å above the best plane. The Pd atom is coordinated by four nitrogen atoms in a square planar fashion, with significant widening of the Npz–Pd–Npz angle (118.5 °). The two Cu^{2+} ions occupy the bidentate sites of **L** and are bridged by OH and acetate, with a Cu···Cu distance of 3.17 Å. Cu coordination is highly distorted from square planar, apparently the cavity of $[Pd(L\text{-}4H)]^{2-}$ is too small for incorporation of two Cu^{2+} ions in a regular square planar coordination. Although the bonding parameters of Pd(II) and Pt(II) in their tetraaza square planar complexes are very similar,[8] the Pt^{2+} complex of **L** coordinates only one Cu^{2+} ion, possibly because Pt^{2+} as a third row transition metal is less tolerant to distortions from regular square planar coordination (such as widening of one angle to > 118°) than Pd^{2+}.



Scheme 1

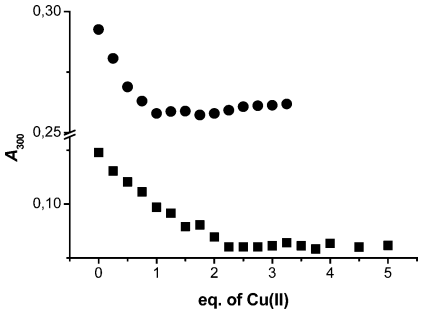


Fig. 1 Decrease of 300 nm absorbance on photometric titration of **1** (10^{-5} M) (■), and **2** (10^{-5} M) (●) with copper(II) acetate solution.

† Electronic supplementary information (ESI) available: EPR spectra. See http://www.rsc.org/suppdata/cc/b3/b316225g/

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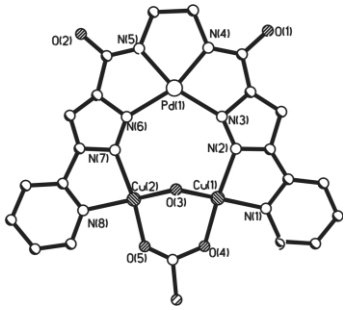
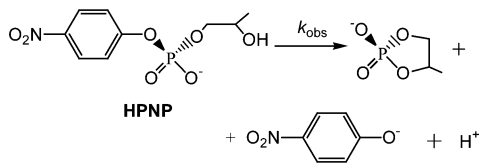


Fig. 2 The molecular structure of $[PdCu_2(L\text{-}4H)(OH)(CH_3COO)]$ with the crystallographic numbering of atoms.

We have studied *in situ* prepared copper complexes of **1** and **2** as catalysts for the cleavage of the activated phosphodiester, 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP), an analogue of RNA. Intermolecular cyclization of this phosphodiester (Scheme 2) was followed photometrically by the 400 nm absorbance of released nitrophenolate. Kinetic studies were performed at varying Cu^{2+} concentrations in a buffered DMSO–water 1 : 1 mixture at pH 7.0, 10^{-4} M complex concentration and 5×10^{-4} M HPNP.

Cleavage rate of HPNP at 0–1 equiv Cu^{2+} is very low which is explained by saturation of in-plane coordination sites of metal ions in both the allosteric (Pd^{2+} or Pt^{2+}) and catalytic site. Further additions of $Cu(NO_3)_2$ do not increase activity of **2**, but substantially that of **1**, with maximum rate at about 3 equiv Cu^{2+} ($k_{obs} \approx 8.0 \times 10^{-5}$ s^{-1}) (Fig. 3). As in our previous studies on dicopper(II) allosteric catalysts,[2a] this can be explained by incomplete complex formation at 2 equiv Cu. This is confirmed by spectrophotometric



Scheme 2 Intramolecular cleavage of the phosphodiester 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP).

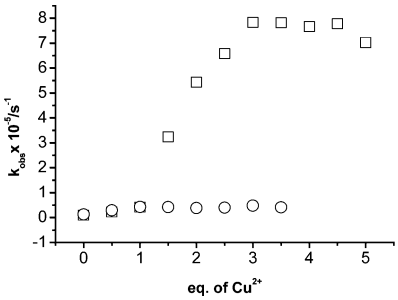


Fig. 3 k_{obs} for cleavage of HPNP (5×10^{-4} M) in solutions containing **1**(□) (10^{-4} M) and **2** (○) (10^{-4} M) and varying copper(II) nitrate concentrations. Buffer 5 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), T = 20 °C. Cleavage by free Cu^{2+} is negligible.

titration of $[Pd(L\text{-}4H)]^{2-}$ with $Cu(NO_3)_2$ in the presence of the inert phosphodiester dimethyl phosphate (5×10^{-4} M) which is apparently less effective in stabilizing $[Cu_2Pd(L\text{-}4H)]^{2+}$ than the better donor acetate. Activity decreases at > 5 equiv Cu, possibly due to competitive complexation of the substrate by free Cu^{2+}.

Acetate (1 equiv) is a strong inhibitor of catalysis, presumably since it competes with the phosphodiester substrate for coordination to the free Cu sites. We suggest double Lewis acid activation[9] of HPNP by bridging coordination to both Cu(II) ions as an important mechanistic contribution to the high reactivity of the dicopper complex.

In conclusion, we describe a new type of allosteric catalyst in which the metal ion coordinated to an allosteric site controls the nuclearity (mono- or dicopper) of a catalytic site. This corresponds to an on/off regulation of catalysis of phosphodiester cleavage since only the dicopper species is catalytically active. Interestingly, this is achieved with allosteric metal ions Pd^{2+} and Pt^{2+} which usually have very similar bonding parameters in their square-planar complexes. Possibly the higher tolerance of Pd^{2+} to distortions of a regular coordination favours incorporation of two Cu^{2+} ions into $[(\mathbf{L}\text{-}4H)M]^{2+}$.

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Notes and references

1 In enzyme catalysis allosteric regulation is the control of enzyme activity by noncovalent modifiers (molecules of ions) which bind to the enzyme at a site other than the active site but alter the conformation of the active site.

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