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Synergic effect of two metal centers in catalytic hydrolysis of methionine-containing peptides promoted by dinuclear palladium(II) hexaazacyclooctadecane complex†

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The species obtained by the reaction of $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ (where $[18]aneN_6$ is 1,4,7,10,13,16-hexaazacyclooctadecane) with $AgBF_4$ have been determined by electrospray ionization mass spectrometry (ESI-MS) to be an equilibrium mixture of three major types of dinuclear Pd(II) complex cations, $[Pd_2(\mu\text{-}O)([18]aneN_6)]^{2+}$, $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$ and $[Pd_2(H_2O)(OH)([18]aneN_6)]^{3+}$, in aqueous solution. The hydroxo-group-bridged one, $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$, is a dominant species, whose crystal structure has been obtained. The crystal structure of $[Pd_2(\mu\text{-}OH)([18]aneN_6)](ClO_4)_3$ shows that each Pd(II) ion in the dinuclear complex is tetra-coordinated by three nitrogen atoms and one hydroxo group bridge in a distorted square configuration. The two Pd(II) ions are 3.09 Å apart from each other. The dinuclear Pd(II) complex cations $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$ and $[Pd_2(H_2O)(OH)([18]aneN_6)]^{3+}$ can efficiently catalyze hydrolysis of the amide bond involving the carbonyl group of methionine in methionine-containing peptides with turnover number of larger than 20. In these hydrolytic reactions, the two Pd(II) ions are synergic; one Pd(II) ion anchors to the side chain of methionine and the other one delivers hydroxo group or aqua ligand to carbonyl carbon of methionine, or acts as a Lewis acid to activate the carbonyl group of methionine, resulting in cleavage of Met–X bond. The binding constant of dinuclear Pd(II) complex cations with AcMet–Gly and AcMet were determined by ^1H NMR titration to be 282 ± 2 M^{-1} and 366 ± 4 M^{-1}, respectively. The relatively low binding constants enable the catalytic cycle and the possible catalytic mechanism is proposed. This is the first artificial mimic of metallopeptidases with two metal active centers.

Introduction

The selective hydrolytic cleavage of peptides and proteins is one of the most important reactions involved in various biological processes.[1] Over the past decade, a variety of metal complexes have been used as "inorganic proteases" to cleave the amide bonds in peptides and proteins.[2–9] Several palladium(II)[10–17] and platinum(II)[16–18] complexes have been found to promote effectively the hydrolysis of peptides and proteins containing methionine and histidine residues through coordination to the side chains. According to the reaction mechanism they are mononuclear model complexes; only one metal ion participates in binding to substrate and activation of amide bond in the vicinity of the binding site. However, many natural metallopeptidases have been discovered to contain a dinuclear metal active center.[19] Bovine lens leucine aminopeptidase contains two zinc ions, 3.0 Å apart from each other.[20] *Aeromonas proteolytica* aminopeptidase contains two zinc ions, with a distance of 3.5 Å.[21] Methionine aminopeptidase from *E. coli* contains two Co(II) ions, with a distance of 2.9 Å.[22] Both metal ions cooperatively participate in substrate binding and activation. Various hydrolytic enzymes for the cleavage of polynucleotides contain multiple metal ions at their active sites.[19,23,24] Dinuclear model complexes have been found more effective than mononuclear ones in hydrolysis of phosphate ester.[25] Therefore, it is necessary to extend the research of artificial metallopeptidases from mononuclear model to dinuclear one, in order to obtain new artificial hydrolytic cleavage reagents for peptides and proteins with high efficiency and large catalytic turnover number.

In this report, the dinuclear complex $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$[26] (where $[18]aneN_6$ is 1,4,7,10,13,16-hexaazacyclooctadecane) was selected as a model compound. There were two advantages of this choice. The first was that this dinuclear complex is well characterized and has high stability,[26] and only one site of coordination for each Pd(II) center can be replaced by side chain of methionine or other donor groups in peptides and proteins. The second was that the distance of two palladium(II) ions in this complex is 3.04 Å, similar to that of 3–5 Å found in natural metallopeptidases.[23] It would be expected that the synergic effect of two palladiums occurs in the activation of substrate promoted by the dinuclear palladium(II) complex. Fortunately, we have found that the hydrolysis of methionine-containing peptides can be catalyzed by the species obtained from $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ treated by $AgBF_4$. To the best of our knowledge, this is the first true dinuclear metal center model for synergic hydrolysis of peptides.

Results and discussion

Composition of dinuclear Pd(II) complex in solution

An aqueous solution, obtained from $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ *via* removal of AgCl formed by adding $AgBF_4$ at pH 1.2 (adjusted by $HClO_4$), was detected by electrospray ionization mass spectrometry (ESI-MS). As shown in Fig. 1, four major peaks appear at m/z 244.0, 287.9, 293.9 and 302.9 with

† Electronic supplementary information (ESI) available: Variation of the ^1H NMR chemical shift of the CH_3S group with molar ratio of Pd(II) complex to peptide. See http://www.rsc.org/suppdata/dt/b5/b500210a/

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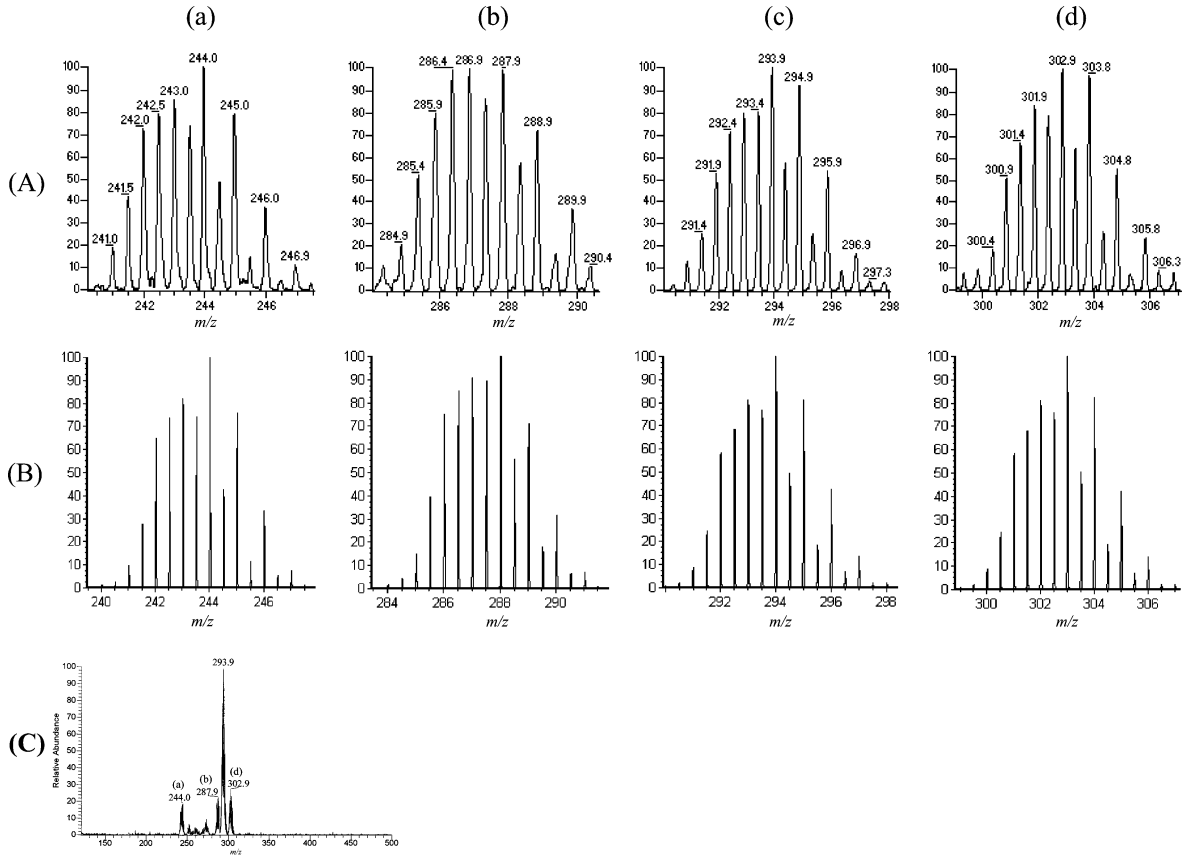
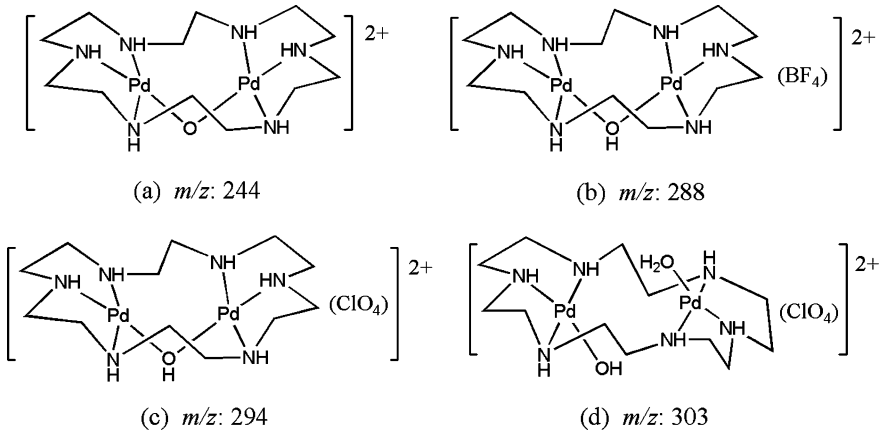


Fig. 1 ESI mass spectrum of an aqueous solution obtained from $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ through removal of AgCl formed by adding $AgBF_4$. (A) High resolution scans of the four major peaks; (B) Isotope distribution patterns for (a) $[C_{12}H_{30}N_6OPd_2]^{2+}$, (b) $[C_{12}H_{31}BF_4N_6OPd_2]^{2+}$, (c) $[C_{12}H_{31}ClN_6O_5Pd_2]^{2+}$, (d) $[C_{12}H_{33}ClN_6O_6Pd_2]^{2+}$ calculated by the IsoPro 3.0 program.

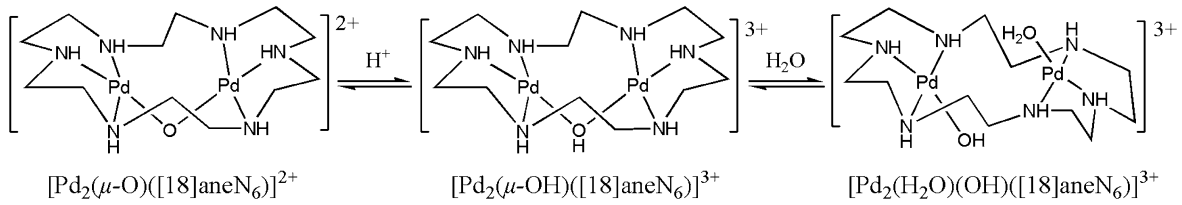


Scheme 1 The major species observed by ESI-MS.

doubly positive charges. The spectrum simulations based on corresponding composition given in Scheme 1 fit to each of the higher-resolution spectra very well. On the basis of ESI-MS measurement, three major types of dinuclear Pd(II) complex cations, $[Pd_2(\mu\text{-}O)([18]aneN_6)]^{2+}$, $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$ and $[Pd_2(H_2O)(OH)([18]aneN_6)]^{3+}$, exist in aqueous solution and are in equilibrium, as shown in Scheme 2. It is worth noting that the existence of $[Pd_2(\mu\text{-}O)([18]aneN_6)]^{2+}$ may be favored under the ESI-MS conditions over that in aqueous solution, since charge reduction of the multiply charged ion is a common phenomenon in ESI-MS.[27] Judging by the relative ESI-MS intensities, the dominant species is the hydroxo-group-bridged one, the crystal structure of which has been obtained. The other minor peaks appeared at *m/z* 252.9, 260.3, 268.9, 276.1 are attributed to $[Pd_2(OH)_2([18]aneN_6)]^{2+}$, $[Pd_2(OH)(OCH_3)([18]aneN_6)]^{2+}$, $[Pd_2(OH)(OCH_3)([18]aneN_6)(H_2O)]^{2+}$, $[Pd_2(OH)(OCH_3)([18]aneN_6)(CH_3OH)]^{2+}$, respectively. The last three complexes formed under ESI-MS experimental conditions.

Crystal structure of $[Pd_2(\mu\text{-}OH)([18]aneN_6)](ClO_4)_3$

To gain further insight into the structure of the species formed in solution, the aqueous solution prepared at the same condition of ESI-MS measurements was crystalized at 4 °C, the single crystals of $[Pd_2(\mu\text{-}OH)([18]aneN_6)](ClO_4)_3$ were obtained and confirmed by X-ray crystallographic analysis. The structure of $[Pd_2(\mu\text{-}OH)([18]aneN_6)](ClO_4)_3$ consists of a dinuclear cation of $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$ and three ordered counterpart anions of perchlorate. Fig. 2 shows a drawing of the molecular structure of the cationic complex. The selected bond lengths and bond angles and crystallographic data are listed in Table 1. Each palladium ion in the dinuclear complex is tetra-coordinated by



Scheme 2 An equilibrium among the dinuclear Pd(II) complex cations in aqueous solution.

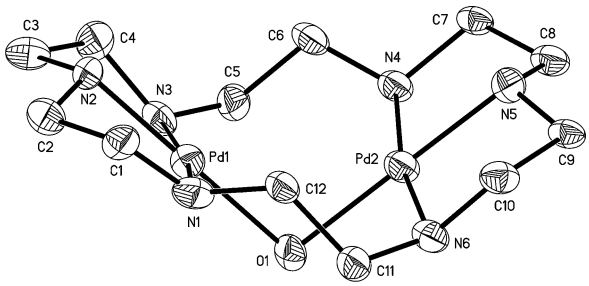


Fig. 2 Drawing of the dinuclear cation of $[Pd_2(\mu\text{-OH})([18]aneN_6)](ClO_4)_3$ with 30% thermal displacement ellipsoids. All hydrogen atoms and three ordered perchlorate anions are omitted for clarity.

Table 1 Selected bond lengths/Å and angles/° for $[Pd_2(\mu\text{-OH})([18]aneN_6)](ClO_4)_3$[a]

Pd1–O1	2.087(6)	Pd2–O1	2.076(6)
Pd1–N1	2.085(9)	Pd2–N4	2.094(9)
Pd1–N2	1.996(8)	Pd2–N5	2.002(8)
Pd1–N3	2.014(9)	Pd2–N6	2.041(9)
O1–Pd1–N1	99.0(3)	O1–Pd2–N4	96.0(3)
O1–Pd1–N2	177.3(3)	O1–Pd2–N5	177.7(3)
O1–Pd1–N3	91.8(3)	O1–Pd2–N6	93.3(3)
N1–Pd1–N2	83.7(4)	N4–Pd2–N5	86.2(3)
N1–Pd1–N3	169.0(4)	N4–Pd2–N6	170.2(3)
N2–Pd1–N3	85.5(4)	N5–Pd2–N6	84.6(4)
		Pd1–O1–Pd2	95.9(3)

[a] $C_{12}H_{31}Cl_3N_6O_{13}Pd_2$, $M = 786.58$, crystal system = orthorhombic, space group = $P2_12_12_1$ (no. 19), $a = 9.5368(7)$ Å, $b = 13.202(1)$ Å, $c = 19.890(2)$ Å, $U = 2504.2(3)$ Å^3, $T = 293(2)$ K, $Z = 4$, μ(Mo Kα) = 1.8 mm^{-1}, No. of reflections collected = 13483, No. of unique data (R_{int}) = 2791 (0.051). The final R indices [$I > 2\sigma(I)$]: $R_1 = 0.0472$; $wR_2 = 0.1195$, R indices (all data): $R_1 = 0.0608$; $wR_2 = 0.1244$.

three nitrogen atoms and one hydroxo group bridge in a distorted square configuration. The two palladium ions are 3.091(1) Å apart from each other. The angle between the coordination planes of Pd1 (containing Pd1, N1, N2, N3, O1) and Pd2 (containing Pd2, N4, N5, N6, O1) is 85.7(2)°. The conformation of the cationic complex is twist-boat-like.

Hydroxide-ion-bridged dinuclear Pd(II) complexes have been known for many years.[28] However, the dinuclear Pd(II) complex of polyazacycloalkane with a bridging hydroxo group is reported here for the first time. As Table 1 shows, the Pd–N distance of 1.996 to 2.094 Å in the cationic complex are comparable to that of 1.98–2.174 Å in $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$,[26] $[Pd_2([18]aneN_6)Br_2]Br_2(H_2O)_4$,[29] $[Pd_2([20]aneN_6)Br_2]Br_2(H_2O)$,[29] and $[Pd_2([24]aneN_8)](ClO_4)_4$[30] complexes. The Pd–O distances are also comparable to those in other hydroxo-group-bridged dinuclear Pd(II) complexes, such as $(NBu_4)_2[(C_6F_5)_2Pd(\mu\text{-OH})_2Pd(C_6F_5)_2]$[31] and $[(Me_3P)_2Pd(\mu\text{-OH})_2Pd(PMe_3)_2](CF_3SO_3)_2$.[32]

It is noted that the structure of $[Pd_2(\mu\text{-OH})([18]aneN_6)](ClO_4)_3$ determined corresponds exactly to the predominant species observed in ESI-MS measurement in the same preparation conditions of pH 1.2. On the basis of the pK_a value of 3.0 for mononuclear Pd(II) aqua complexes,[33] in our experimental condition of pH ~ 1, the predominant species would be aqua-ligand-coordinated, instead of hydroxo-group-bridged or coordinated. However, in fact, all species observed by ESI-MS measurements and crystallographic analysis showed hydroxo bridges or oxygen bridges or hydroxo coordination. This means that the dinuclear Pd(II) complex has a strong tendency towards formation of a hydroxo species, even in more acidic solution. The hydrolytic reaction is very common for the hydrated metal ions with high oxidation state. Therefore, it is reasonable to believe that the formation of a hydroxo species of the dinuclear Pd(II) complex occurring in more acidic solution is probably due to the high positive charges of the cationic complex. These hydroxylated species observed are realistic ones present in the solution.

Interaction of peptides with dinuclear Pd(II) complex cations

A solution of dinuclear Pd(II) complex obtained by treatment of $AgBF_4$ for removal of chloride ions from $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ was mixed with methionine-containing peptide in a molar ratio of 1:1 at pH 1.1 and room temperature for 10 min, and then measured by ESI-MS. When AcMet–Gly was taken as an example, besides the peaks at m/z 244.0, 287.9 and 293.9 present in Fig. 1, a new peak at m/z 367.9 separated by 0.5 m/z appeared and was assigned to $[Pd_2([18]aneN_6)(OH)(AcMet–Gly)–H]^{2+}$ based on a high-resolution scan and simulation of the isotope distribution pattern. The species, as is shown in Fig. 3, was formed by interaction of AcMet–Gly either with $[Pd_2(\mu\text{-OH})([18]aneN_6)]^{3+}$ *via* attack of AcMet–Gly to one of the two Pd(II) centers, followed by breaking the Pd–OH bond, or with $[Pd_2(H_2O)(OH)([18]aneN_6)]^{2+}$ *via* substitution of the aqua ligand.

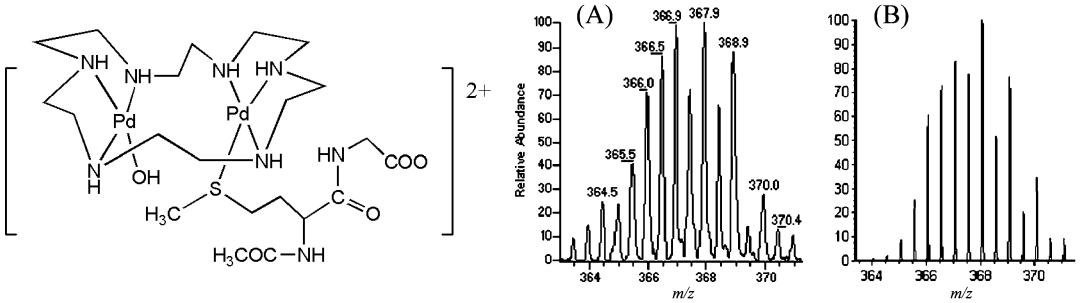


Fig. 3 Assignment for ESI-MS peak at m/z 367.9. (A) High-resolution scan; (B) Isotope distribution patterns for $[C_{21}H_{46}N_8O_5SPd_2]^{2+}$ calculated by the IsoPro 3.0 program.

Attachment of peptides to the dinuclear Pd(II) complex cations was also observed in ^{1}H NMR spectra. The initial coordination of Pd(II) to side chain of methionine-containing peptides was accompanied by migration of chemical shift of the CH_3S towards downfield, as summarized in Table S1 (in the electronic supplementary information (ESI)†). Furthermore, as shown in Table S1, the chemical shift of CH_3S varies with the molar ratio of the complex to substrate. In ^{1}H NMR spectrum, only one signal of the CH_3S appeared in the range of molar ratio varied indicates a rapid exchange in the NMR time scale between the free and binding AcMet-X at room temperature. A detail NMR titration for AcMet–Gly was made, and the result shown in Fig. 4 confirmed again that AcMet–Gly is coordinated to the dinuclear Pd(II) complex in 1:1 stoichiometry. According to the equation in ref. 34 the binding constant, K, was calculated to be 282 ± 2 M^{-1}, which is relatively low.

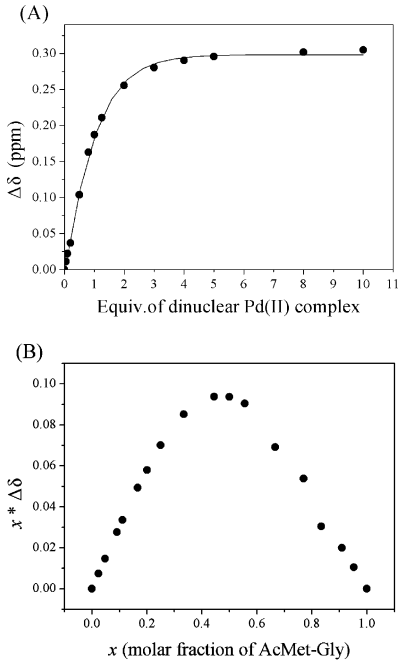


Fig. 4 (A) NMR titration curve in D_2O. (B) Jobs plot.

Catalytic hydrolysis of peptides promoted by dinuclear Pd(II) complex cations

As discussed above, the composition of the dinuclear Pd(II) complex-anchored by peptide is well characterized (See Fig. 3) by means of combination of ESI-MS measurement and ^{1}H NMR titration. The hydrolysis reaction occurred in the complex $[Pd_2([18]aneN_6)(OH)(AcMet\text{-}Gly)]^{3+}$ and was monitored by ^{1}H NMR. The amide bond at carboxyl terminus of methionine was hydrolyzed, as reported previously.[10–12] Table 2 gives the observed rate constants, k_{obs} for different methionine-containing peptides. As shown in Table 2, the rate constant decreases in some extent as the molar excess of the dipeptide over the promoter increases. Although the reaction is not fast enough, it is significant in comparison with control experiment. At the same experimental condition, the observed rate constant for dipeptide hydrolysis in the absence of the dinuclear Pd(II) complex was determined to be 0.82 × 10^{-3} h^{-1}, 21 times slower than the lowest one in the presence of the dinuclear Pd(II) complex. Thereby, the achievement of the catalysis is important, though the turnover number of 20 is relatively small.

The effect of pH was examined with the substrate AcMet–Gly. As shown in Table 3, the observed rate constant for hydrolysis of Met–Gly bond increases as the solution is more acidic. Previous studies reported that the acidic solution can enhance the hydrolytic rate of amide bond through inhibiting the oligomerization of mononuclear Pd(II)-aqua complexes, caused by hydroxo-bridging.[11] In this study, the acidic solution may be associated with breaking of hydroxo-bridging in $[Pd_2(\mu\text{-}O)([18]aneN_6)]^{2+}$ and $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$, favorable for binding of substrate to the dinuclear Pd(II) complex.

The effect of temperature on the hydrolytic reaction was also examined with the substrate AcMet-Gly. The data are given in Table 4, and the apparent activation enthalpy, $\Delta H^{\ddagger}$ and activation entropy, $\Delta S^{\ddagger}$ are 75.0 ± 3.2 kJ mol^{-1} and −103.4 ± 9.9 J K^{-1} mol^{-1} respectively, calculated according to the Eyring equation.

As known from previous studies, $[Pd(dien)(H_2O)]^{2+}$ and $[Pt(dien)(H_2O)]^{2+}$ is inactive for the hydrolysis of amide bond, due to the absence of extra aqua ligands after its binding to substrate.[12,17] However, in the complex $[Pd_2([18]aneN_6)(OH)(AcMet–Gly)]^{3+}$, the peptide coordinates to one Pd(II) center, and the other Pd(II) center remains to be coordinated by the hydroxo group or an aqua ligand. Therefore, it is evident that the amide bond of Met–X in AcMet–X is hydrolyzed by the two Pd(II) centers.

Table 2 Catalytic hydrolysis of the Met–X bond in AcMet–X, promoted by dinuclear Pd(II) complex cations at pH* ~ 1.0, and 50 ± 0.5 °C

Substrate	Molar ratio, promoter : substrate	$10^3 k_{obs}/h^{-1}$
AcMet–Gly	1 : 1	74.9 ± 0.5
	0.5 : 1	65.0 ± 0.5
	0.2 : 1	51.9 ± 0.4
	0.1 : 1	31.7 ± 0.2
	0.05 : 1	17.3 ± 0.1
	0 : 1	0.82 ± 0.01[a]
AcMet–Ala	1 : 1	38.1 ± 0.2
	0.5 : 1	33.1 ± 0.2
	0.2 : 1	25.1 ± 0.1
	0.1 : 1	15.9 ± 0.1
	0.05 : 1	9.2 ± 0.1[a]
AcMet–Ala–Ser	1 : 1	35.1 ± 0.3
	0.5 : 1	32.6 ± 0.2
	0.2 : 1	23.4 ± 0.2
	0.1 : 1	14.5 ± 0.1
	0.05 : 1	6.3 ± 0.1[a]
AcMet–Pro	1 : 1	37.0 ± 0.2
	0.5 : 1	32.9 ± 0.3
	0.2 : 1	24.1 ± 0.2
	0.1 : 1	15.0 ± 0.1
	0.05 : 1	7.6 ± 0.1[a]

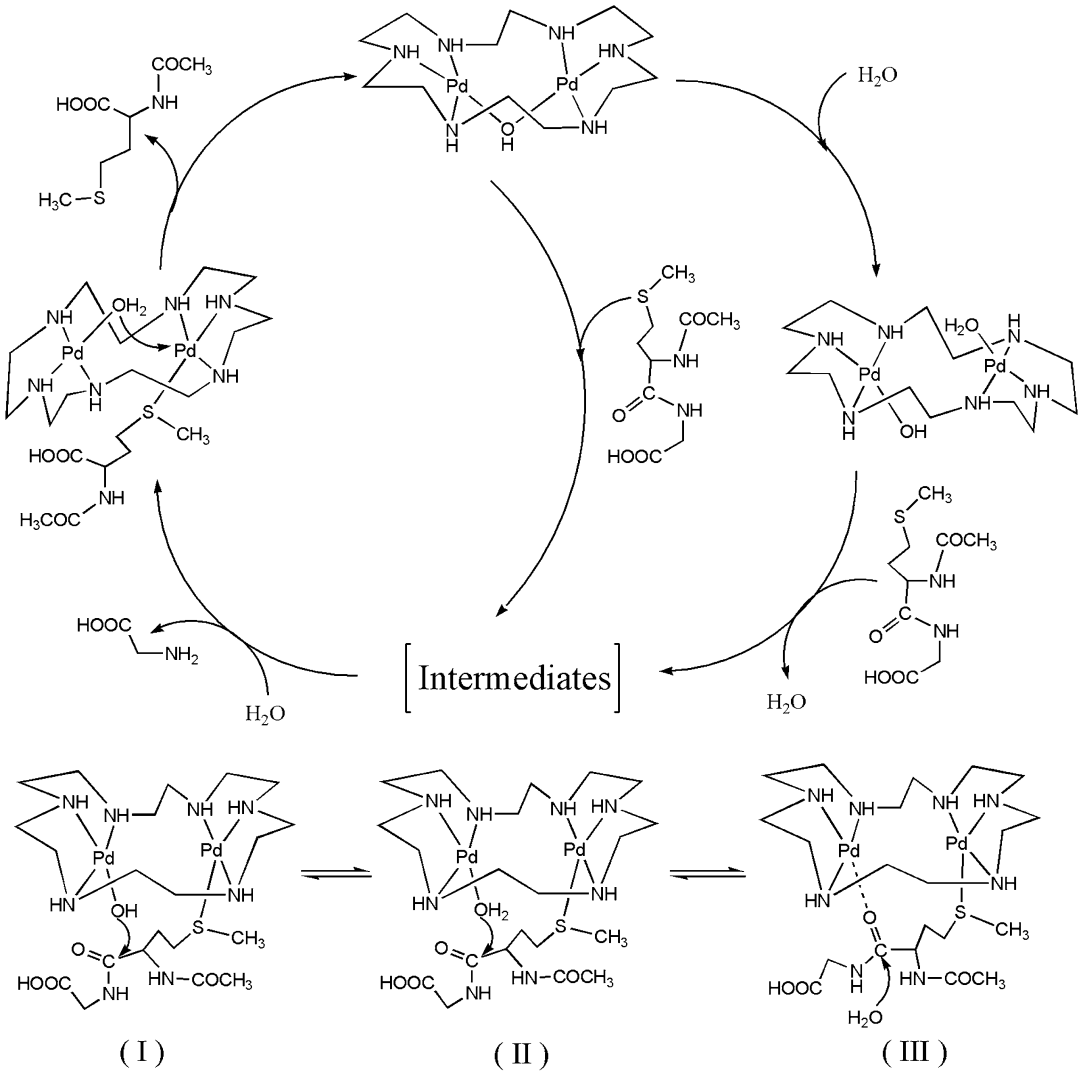
[a] Followed for less than three half-lives.

Table 3 Effect of pH on hydrolysis of Met–Gly bond in AcMet–Gly, promoted by dinuclear Pd(II) complex cations, at 50 ± 0.5 °C

Molar ratio, promoter : substrate	pH*	$10^3 k_{obs}/h^{-1}$
1 : 1	2.01	23.7 ± 0.2
1 : 1	1.65	34.6 ± 0.3
1 : 1	0.98	74.9 ± 0.5

Table 4 Effect of temperature on hydrolysis of Met–Gly bond in AcMet–Gly, promoted by dinuclear Pd(II) complex cations, at pH* ~ 1.0

Molar ratio, promoter : substrate	Temperature/°C	$10^3 k_{obs}/h^{-1}$
1:1	40	27.9 ± 0.1
1:1	50	74.9 ± 0.5
1:1	60	167.5 ± 1.6



Scheme 3 Proposed catalytic cycle for hydrolysis of AcMet–X (herein X = Gly) promoted by dinuclear Pd(II) complex. Three intermediates are involved in cleavage of Met–Gly bond *via* internal delivery of a hydroxy (I) or an aqua ligand (II) or Lewis acid activation (III).

The catalytic cycle

As mentioned above, AcMet–Gly coordinates to the dinuclear Pd(II) complex with a relatively low binding constant, and the hydrolytic reaction occurs in the complex $[Pd_2([18]aneN_6)(OH)(AcMet–Gly)]^{3+}$ with a turnover number of at least 20. This means that these hydrolytic reactions are efficiently catalyzed by the dinuclear Pd(II) complex. Importantly, these reactions can be taken as an artificial mimic of metallopeptidases with two metal active centers, though the hydrolytic reactions promoted by the model complex are not fast enough at the moment. A possible catalytic cycle is proposed in Scheme 3. In the catalytic cycle, both $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$ and $[Pd_2(H_2O)(OH)([18]aneN_6)]^{3+}$ are able to be coordinated by one AcMet–Gly *via* side chain of methionine residue to form intermediate complexes. The cleavage of Met–Gly bond occurs in three possible ways. A hydroxo group or an aqua ligand that coordinates to another Pd(II) center attacks the carbonyl carbon of methionine, as shown in Scheme 3(I) and (II). Lewis acid activation of Met–Gly bond is also involved in the Scheme 3(III). The release of AcMet from the Pd(II) center is the key step for completing the catalytic cycle. The binding constant of AcMet to the dinuclear Pd(II) complex was also determined to be 366 ± 4 M^{-1}. Again, the relatively low binding constant makes the catalytic cycle possible. After completing the hydrolytic reaction monitored by ^1H NMR, the NMR tube was kept at 4 °C for crystallization. The crystals obtained has the same structure as given in Fig. 2, further confirming that the complex of $[Pd_2(\mu\text{-}OH)([18]aneN_6)](ClO_4)_3$ is stable and can be recycle.

Conclusion

In this report, a well defined dinuclear Pd(II) complex of $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ was chosen as a precursor to mimic metallopeptidases with two metal active centers. In contrast to mononuclear Pd(II) complex of $[Pd(dien)(H_2O)]^{2+}$ that is inactive towards hydrolysis of amide bond in peptides, the hydrolysis of methionine-containing peptides can be catalyzed by the dinuclear Pd(II) complex cations of $[Pd_2(\mu\text{-}OH)([18]aneN_6)]^{3+}$ and $[Pd_2(H_2O)(OH)([18]aneN_6)]^{3+}$ with turnover number of at least 20. In the complex of $[Pd_2(OH)([18]aneN_6)(AcMet\text{-}X)]^{3+}$, the amide bond at carboxyl terminus of methionine is cleaved *via* synergism of two metal centers and internal delivery or Lewis acid activation mechanism or both. The low binding ability of substrates and leaving products to one Pd(II) center of the dinuclear Pd(II) complex makes the catalytic cycle proceeding smoothly. Although the catalytic reaction is not fast enough currently, this finding is promising and provides a clue in the pursuit of the final goal of artificial metallopeptidases with two metal active centers.

Experimental procedures

Chemicals

Double distilled water was used for preparation of solutions. The D_2O, anhydrous $AgBF_4$, and $K_2[PdCl_4]$ were obtained from Aldrich. 1,4,7,10,13,16-Hexaazacyclooctadecane ([18]aneN_6) was obtained from Fluka Chemie. Methionyl glycine (Met–Gly), methionyl alanine (Met–Ala), and methionyl alanyl serine (Met–Ala–Ser) were obtained from Sigma. The peptides used were acetylated based on a published procedure.[16] Acetyl methionyl proline (AcMet–Pro) was synthesized by the GL Biochem (Shanghai) Ltd, and its purity was checked by HPLC. ^{1}H NMR spectrum of the AcMet–Pro in D_2O showed the following principal δ values: 3.92–3.81 and 3.78–3.68 (m, CH_2N of Pro), 2.12 (s, CH_3S), 2.01 (s, CH_3CO). The complex $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ was prepared by a published procedure,[26] the chloride ions in which were removed by the following procedure. To a solution of 15.0 mg (0.02 mmol) $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ in 0.70 mL water were added 0.060 mL of 1.50 M $HClO_4$ and 0.100 mL of 0.400 M $AgBF_4$. Water was then added to adjust the total volume up to 1.00 mL. This mixture solution was stirred for 12 h at 35 °C. A white precipitate of AgCl formed was removed by centrifugation. All the above procedures were performed in the dark. The pH value of the resulting solution was 1.2. ^{1}H NMR spectrum of the species prepared in D_2O showed the following principal δ values: 3.66–3.49(m), 3.41–3.19(m) and 3.13–2.52(m). The species in H_2O or D_2O were freshly prepared prior to use.

Measurements

Routine ^{1}H NMR spectra in D_2O with sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal reference, were recorded with Bruker Avance 300 NMR spectrometers. pH was measured with an Orion 901 instrument and a Phoenix Ag–AgCl reference electrode. The uncorrected values in D_2O were designated pH*. Electrospray ionization mass spectra were recorded using an LCQ electrospray mass spectrometer (ESI-MS, Finnigan) by loading 1.0 μL solution into the injection valve of the LCQ unit and then injecting into the mobile phase solution (50% of aqueous methanol), which was carried through the electrospray interface into the mass analyzer at a rate of 200 μL min^{-1}. The voltage employed at the electrospray needles was 4.5 kV, and the capillary was heated to 200 °C. Positive ion mass spectra were obtained. High-resolution scans and simulations of isotope distribution patterns using IsoPro 3.0 program were performed for each of the major species detected.

X-Ray crystallography

0.5 mL of $NaClO_4$ saturated solution was added to an aqueous solution of pH 1.2 prepared by removal of chloride ions from $[Pd_2([18]aneN_6)Cl_2](ClO_4)_2$ according to above described procedure. Single crystals suitable for X-ray crystallographic analysis were obtained by keeping the aqueous solution at 4 °C for *ca.* 10 d. These single crystals can also be obtained from NMR tube keeping at 4 °C for *ca.* 20 d after ^{1}H NMR monitoring hydrolytic kinetics. A single crystal was mounted on a glass fiber. The intensity data for the crystal was collected on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (0.71073 Å) at room temperature in ϕ–ω scans. The Saint and SADABS programs carried out data integration and empirical absorption corrections.[35] The structure was solved by direct method and refined on F^2 using SHELXTL suite of program.[35] All non-hydrogen atoms were anisotropically refined by full-matrix least-squares methods. All hydrogen atoms were geometrically generated and isotropically refined using a riding model.

CCDC reference number 253854. See http://www.rsc.org/suppdata/dt/b5/b500210a/ for crystallographic data in CIF or other electronic format.

Kinetics of hydrolysis

The concentration of the freshly prepared aqueous solution of chloride-free dinuclear palladium(II) complex was 20.0 mM at pH* *ca.* 1.2. A variable amount of above solution of palladium(II) complex, 120 μL AcMet–X (75.0 mM, X is Gly, Ala, Ala–Ser and Pro) and 10.0 μL DSS (100 mM), all as D_2O solutions, were mixed in an NMR tube. The pH* value was adjusted with 1.5 M $HClO_4$ in D_2O at the beginning, and was measured again at the end of reaction, the difference was less than 0.10 and the final value was reported. The total volume was adjusted with D_2O to 600 μL. The final concentration of peptide was 15.0 mM. Acquisition of ^{1}H NMR spectra began as soon as possible, and 32 scans were taken at each time. The sample temperatures were kept at 40, 50, or 60 ± 0.5 °C. Resonances of the following groups allow clearly monitoring of peptides and hydrolysis products: CH_2 of Gly, CH_3 of Ala, CH_2N of Pro and CH_3 of Ala–Ser. The concentrations of the peptides and of the hydrolysis products were determined, with an estimated error of ±5%, from the known initial concentrations of peptide and from the integrated resonances of the leaving group and the DSS internal standard. The hydrolysis products were identified by addition of pure Gly, Ala, Ala–Ser or Pro to the corresponding reaction mixture, only enhancing the ^{1}H NMR signals of hydrolysis products, without new signal appeared. First-order logarithmic plots of substrate concentration or hydrolysis product concentration *versus* time were linear for three half-lives. A few slow reactions were monitored for less than three half-lives. A typical kinetic plot was consisted of 10–18 points, and correlation coefficients were higher than 0.99.

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34 $a/(\delta_{obs} - \delta_0) = (1/K)[1/C_0(\delta_{max} - \delta_{obs})] + 1/(\delta_{max} - \delta_0)$, where C_0 is the initial concentration of dinuclear Pd(II) complex, mol L^{-1}; a is the initial concentration ratio of peptide to the complex; δ_0 is the ^{1}H NMR chemical shift of the CH_3S for free peptide; δ_{max} is the ^{1}H NMR chemical shift of the CH_3S for the mixed solution with much excess of the complex over peptide; δ_{obs} is the ^{1}H NMR chemical shift of the CH_3S in different molar ratio of dinuclear Pd(II) complex to peptide. The binding constant, K, can be obtained by self-consistent calculation with respect to δ_{max}.

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